THE ROLE OF KININS IN INFLAMMATROY PAIN AND THEIR MODULATION BY OTHER INFLAMMATORY MEDIATORS.

David C. Kelly

Doctor of Philosophy
University of Edinburgh
1998

DECLARATION

I declare that this thesis was composed entirely by me and represents all my own work except for those procedures listed below.

In a minority of the electrophysiological experiments studying the effects of mediators on neural discharge from C-fibres innervating the ankle or knee joint. These were carried out in collabaration with Dr. A.U.R. Asghar and Mr. C. Marr. In such cases either surgery or injection of drugs was carried out by these persons.

D.C. Kelly

ACKOWLEDGEMENTS

I would like to thank my Ph.D. supervisors, Dr. D. S. McQueen and Dr M. Perkins, for their help, encouragement and patience throughout my period of study. I would also like to thank the staff at the University of Edinburgh and the Novartis Institute for Medical Sciences. In particular I would like to thank Dr Aziz Asghar and Charlie Marr for their help and technical assistance at the outset of my Ph.D and all of the Department of Pharmacology at Novartis for helping me to keep sane during the write up of my thesis and Andrew Davis for his helpful discussions. Finally I would like to thank all my friends and family for their friendship and encouragement.

ABSTRACT

This thesis tests the hypothesis that certain inflammatory mediators, including kinins and cytokines are involved in sensitisation of peripheral nociceptors and contribute to the hyperalgesia associated with inflammatory conditions. These studies involved the development of a new model for recording from C-fibres innervating the rat knee joint and determined the role of these substances and their interactions in modulating neural discharge from articular nociceptors. Complementary behavioural studies were carried out to assess the role of these mediators in thermal and mechanical hyperalgesia.

Recordings from C-fibres innervating the medial side of the rat knee joint revealed that Bk induced an increase in neural discharge in normal and arthritic knee joints which was blocked by a kinin B_2 receptor antagonist, icatibant. desArg^9-Bk was ineffective in normal joints, however in arthritic joints desArg^9-Bk induced an increase in neural discharge in C-fibres which was mediated via kinin B_1 receptors, as it was reduced by desArg^{9}leu^8-Bk, but not icatibant.

IL-1β injected into the knee joint induced an increase in neural discharge from C-fibres which was blocked by concomitant injection of IL-1ra. This was consistent with behavioural studies where local injection of IL-1β induced a mechanical and thermal hyperalgesia. IL-1β also enhanced responses mediated by Bk in C-fibres and in mechanical hyperalgesia. In the mechanical hyperalgesia model the enhanced responses to Bk were blocked by both desArg^{9}leu^8-Bk and icatibant. IL-1β was also shown to be involved in inducing a desArg^9-Bk-mediated increase in neural discharge in C-fibres and a desArg^9-Bk-mediated behavioural hyperalgesia (mechanical and thermal). The desArg^9-Bk mediated hyperalgesia was blocked by desArg^{9}leu^8-Bk, but not icatibant.

Pre-treatment with indomethacin blocks IL-1β-induced increase in neural discharge and behavioural hyperalgesia. Indomethacin also blocks IL-1β-induced enhancement of Bk-mediated increase in neural discharge from C-fibres and IL-1β-induced

enhancement of Bk-mediated mechanical hyperalgesia. Although indomethacin blocked the kinin-B_1-mediated increase in neural discharge induced by IL-1β, indomethacin did not reduce the kinin B_1-mediated mechanical hyperalgesia induced by IL-1β and only blocked the kinin B_1-mediated thermal hyperalgesia induced by IL-1β for one hour.

These studies also considered the involvement of mediators which are increased during inflammation in modulation of C-fibre activity and behavioural hyperalgesia. The role of endogenous nitric oxide in modulating neural discharge of C-fibres was studied in normal and arthritic ankle joints. L-NAME, a nitric oxide synthase inhibitor, induced an increase in neural discharge from articular C-fibres in both normal and arthritic ankle joints and increased Bk-mediated increase in neural discharge in arthritic ankle joints. These effects were reduced by L-Arginine, a nitric oxide precursor. L-Arginine itself reduced Bk-mediated increase in neural discharge in both normal and arthritic ankle joints. The role of two 'anti-inflammatory' cytokines (IL-4 and IL-10) in modulating behavioural hyperalgesia was also studied. IL-4 and IL-10 reduced IL-1β-induced mechanical hyperalgesia and thermal hyperalgesia, however the duration of action of IL-10 (less than 2h) was shorter than IL-4 (at least 5h) in the thermal hyperalgesia model. IL-4 and IL-10 also blocked IL-1β-induced enhancement of Bk-mediated and $desArg^9$-Bk-mediated mechanical and thermal hyperalgesia, however once again, the duration of action of IL-10 (less than 2h) was shorter than IL-4 (at least 5h).

In conclusion, the results illustrate a role for both kinin B_2 and B_1 receptors in modulating neural discharge from knee joint C-fibres and hyperalgesia in inflammation. IL-1β is involved in modulating neural discharge from articular C-fibres and modulation of kinin-mediated modulation of neural discharge which appears to involve generation of prostanoids in the case of enhancement of Bk-mediated responses. These results also highlight a role for IL-4, IL-10 and nitric oxide as mediators which potentially limit the hyperalgesia associated with inflammation. Overall, the present results add further to our knowledge and understanding of the

peripheral mechanisms involved in inflammatory pain.

Index: List of Figures

1. INTRODUCTION.

1.1 Pain and Nociception

The sensation of pain is the body's conscious appreciation of stimuli of such an intensity or nature that they threaten the integrity of tissues. The existence of a neural mechanism that detects potential tissue damage or injury was postulated at the beginning of the century (Sherrington, 1906), based on this concept these pain sensors were termed nociceptors. Following this, several groups identified a sub-group of primary afferents in the skin, muscles and joints which respond to mechanical and thermal stimuli outside the normal physiological range (Bessou & Perl, 1969; Van Hees & Gybels, 1972; Mense, 1985; Schaible & Schmidt, 1984). Since these extensive studies, Sherrington's definition has been updated. Many nociceptors are activated by agents, including kinins which do not necessarily cause tissue damage and can act for long periods when released endogenously. Thus, pain exists long after the potentially damaging stimulus has been removed and it has since been suggested that pain acts as an 'adjunct to protective reflexes' (McMahon & Koltzenburg, 1990). Nociceptors have been classified according to: their response to different modalities of intense stimuli, conduction velocity (CV), and differences in their characteristic response to chemical stimuli (Raja *et al.*, 1988). Although nociceptors are present in a variety of tissues and organs, including skin, muscle, eye etc, the main focus of the present study is on nociceptors innervating articular joints.

In the naive knee joint these have been categorised into the following four groups (Schaible & Schmidt., 1983):

Group I : Nerves excited by innocuous movement (CV: $> 60\ ms^{-1}$).

Group II : Nerves only weakly excited by innocuous movement, while noxious movement leads to pronounced neural discharge(CV: 21 - $60ms^{-1}$).

Group III : Nerves that respond consistently only to noxious join movements (CV: 2.5 - 20 ms^{-1}).

Group IV : Nerves that cannot be excited by any joint movement (CV: $< 2.5\ ms^{-1}$).

The majority (70%) of thin myelinated (Aδ-) and unmyelinated (C-) fibres belong to groups III and IV (Schaible & Grubb, 1993). The discovery that capsaicin, a derivative of capsicum (red peppers) could selectively activate C-fibres has been of great use in the study of pain, allowing the selective stimulation, and therefore identification of nociceptors (Szolcsanyi *et al.*, 1988).

Pain can be classified into two distinct types. The first is protective or physiological pain, which is elicited by intense stimuli and serves to protect against potential damage to the tissue. The second is clinical pain, which accompanies an underlying pathology. For example, pain arises due to tissue injury in the case of inflammatory pain, or as a consequence of disturbances to the nervous system in the case of neuropathic pain (see Woolf, 1987). Treatment of these two types of clinical pain also highlights differences. Conventional analgesics including opiates and NSAIDs are, in general, ineffective or show reduced efficacy in neuropathic pain when compared to inflammatory pain (for review see Fields, 1994).

1.1.1 Hyperalgesia

Hyperalgesia is defined as 'a state of increased pain sensation induced either by noxious or ordinarily non-noxious stimulation of peripheral tissue' and is characterised by increased sensitivity to mechanical or thermal stimuli (Hardy *et al.*, 1952). This can be further subdivided into primary hyperalgesia which occurs at the site of injury and secondary hyperalgesia which occurs in the surrounding undamaged tissue (Campbell *et al.*, 1989). For example, during a hyperalgesic state groups III and IV afferents become sensitised and are activated by lower mechanical thresholds than normal.

Discovering how nociceptors become sensitised and what produces this sensitisation has been of great interest and advantageous in helping the understanding of hyperalgesia. The fact that hyperalgesia accompanies inflammation and inflammatory diseases (including joint pain in arthritis) has led to the study of inflammatory mediators associated with the sensitisation of nociceptors. There is a myriad of mediators released during inflammation and these mediators have many actions and interactions with each other. It is, therefore, questionable as to the physiological value of studying these mediators separately. However, investigating them individually has advanced the understanding of peripheral hyperalgesia and the sensitisation of nociceptors. Several inflammatory mediators have been identified as having sensitising

effects on nociceptors including bradykinin, prostaglandins, 5-hydroxytryptamine (5-HT) and cytokines (bradykinin and cytokines will be discussed in more detail in the Sections 1.3.1 and 1.3.2).

Prostanoids, in particular PGE_1, PGE_2 and PGI_2, have been shown to be more involved in sensitisation of nociceptors rather than excitation. When injected intra-articularly into the cat knee joint, PGE_1 and PGE_2 sensitised nociceptors to movement of the joint (Heppelmann *et al.*, 1985; Schaible & Schmidt, 1988). Similarly, in the rat ankle joint PGE_2 has been shown to sensitise nociceptors to mechanical stimulation and other algesic agents e.g. bradykinin (Birrell *et al.*, 1991). However, PGI_2 has a much greater role in sensitisation of nociceptors; in the rat ankle joint PGI_2 and the more stable agonist cicaprost sensitised the majority of nociceptors (80 - 90%) to mechanical stimulation (Birrell *et al.*, 1991). 5-HT which is also released during inflammation, mainly from mast cells, has been shown to excite and sensitise group III (43%) and IV (73%) afferents to mechanical stimuli in both the cat knee joint and rat ankle joint (Herbert & Schmidt, 1992; Birrell *et al.*, 1990) probably via $5\text{-}HT_3$ receptors. There is some evidence that leukotrienes (LT's), in particular LTB_4 can also sensitise peripheral nociceptors to mechanical stimulation (Martin *et al.*, 1987). Thus, there is the capacity for a range of inflammatory mediators to sensitise nociceptors resulting in hyperalgesia.

1.1.2 Animal models of pain

Animal models are widely used to further the understanding of pain mechanisms due to the limitations of what can be undertaken in human experiments. The models used can be categorised as either acute or persistent. Acute models include tail flick, hot plate, limb-withdrawal reflex and vocalisation thresholds, and use an exogenously applied stimulus (heat or pressure) to excite nociceptors directly. Persistent models are usually associated with a long lasting or chronic inflammation, which alters the local environment around the nociceptors and maintains a persistent state of hyperalgesia (Rang *et al.*, 1991). Persistent pain models may be more relevant in studying the mechanisms underlying the hyperalgesia associated with chronically painful clinical disorders such as arthritis and gout. In these models animals, respond to stimuli, whether thermal, mechanical or chemical, with an

increased sensitivity.

One animal model which has been used extensively to study the mechanisms underlying chronic pain is the polyarthritic rat, in which arthritis is induced by injection of Freund's complete adjuvant (FCA). Newbould (1963) described an arthritic syndrome induced by intradermal injection of 0.05ml of $5mgml^{-1}$ of dead tubercle bacilli into the plantar surface of the rat hind paw and the subsequent development of arthritis was measured in terms of increase in foot thickness. However, although this produces arthritis in the joints and an associated hyperalgesia (Guilbaud *et al.*, 1985), it is a very severe model and induces a systemic disease which includes skin lesions, destruction of bone and cartilage and impairment of liver function (Coderre & Wall, 1987). Less severe models, as well as being ethically more acceptable, may be more appropriate for nociceptive studies and models have been developed which involve localised administration of substances to elicit inflammation and hyperalgesia. The original method of Newbould (1963) has been developed and modified to induce a localised unilateral arthritis.

Persistent mechanical hyperalgesia can be observed and studied following intra-plantar injection of carrageenin, kaolin and Freund's complete adjuvant using the Randall-Selitto paw withdrawal test (Randall & Selitto, 1957). These agents produce a localised inflammation leading to a decreased withdrawal threshold to mechanical stimuli. To study the mechanisms of pain that mimics the clinical conditions of arthritis, joint pain can be induced in animals by injection of pro-inflammatory agents including urate crystals or Freund's complete adjuvant either around the joint or directly into the joint space. In these models the hyperalgesia which develops is evidenced by a reduced threshold tolerated by movement of the joint or pressure applied to the joint (Coderre & Wall, 1987; Grubb *et al.*, 1988; Grigg *et al.*, 1986; Perkins *et al.*, 1993). Some of these studies are purely behavioural, whereas others involve recording the activity of C-fibres innervating the joints as an indication of pain sensation. The posterior articular nerve (PAN) and medial articular nerve (MAN) which innervate the cat knee joint and the primary articulocutaneous ramus (PACR) which innervates the rat ankle joint have been recorded from for this purpose. In these studies it was observed that there was little or no spontaneous neural discharge in nerves innervating normal joints however, nerves innervating inflamed joints had an ongoing spontaneous neural discharge (Grigg *et al.*, 1986;

Birrell *et al.*, 1990) and were activated with lower mechanical thresholds or usually non-noxious movements.

To study the differences and similarities between mechanical and thermal hyperalgesia a model for studying persistent thermal hyperalgesia model has been developed. Following exposure of the plantar surface of the rat paw to ultra-violet irradiation, the treated paw showed a reduction in latency to radiant heat directed at the plantar surface of the paw (Perkins *et al.*, 1993).

In all these models there is an associated inflammation. To study the role of individual inflammatory mediators in the subsequent hyperalgesia, specific putative inflammatory mediators instead of the more non-specific pro-inflammatory agents may be injected locally. In this way a link may be established between inflammation and hyperalgesia, and therefore advances may be made in the understanding and treatment of pain in the clinic.

1.2 Inflammation.

Inflammation consists of a complex series of events which in general terms may be defined as the response of living tissue to trauma or infection. It comprises four main stages : local vasodilation resulting in increased blood flow to the affected area; contraction of endothelial cells resulting in increased vascular permeability and thus leakage of plasma from the micro-circulation into the surrounding tissue; migration of polymorphonuclear leukocytes to the site of inflammation; and subsequent phagocytosis of immune cells and further release of inflammatory mediators (Evans & Whicher, 1992). To be considered an inflammatory mediator a substance should satisfy the following criteria : it must be present in elevated levels during the inflammatory response; when administered it must induce one or more components of the inflammatory response, and antagonists to the mediator should have anti-inflammatory activity.

1.3 Inflammatory mediators.

There is a plethora of mediators involved in the inflammatory process including histamine, 5-hydroxytryptamine, platelet activating factor (PAF), components of the complement system, prostanoids, leukotrienes, neuropeptides, kinins, cytokines and nitric oxide. They are

involved in a complex series of actions and interactions. However, for the purpose of this thesis I have concentrated on kinins, cytokines, prostanoids and nitric oxide.

1.3.1 Kinins

1.3.1.1 Formation

Kinins are formed by the action of proteolytic enzymes termed kallikreins. There are two different kallikreins, plasma kallikrein and tissue kallikrein, both of which exist as inactive forms called plasma pre-kallikrein and tissue prokallikrein and are synthesised in the liver. Plasma pre-kallikrein, a single chain glycoprotein, which is present in the plasma complexed with high molecular weight (HMW)-kininogen (Mandle *et al.*, 1976) and on the external surface of neutrophils, along with HMW- and low molecular weight kininogen (LMW)-kininogen (Figueroa *et al.*, 1992), is activated by Hageman factor. Hageman factor itself is activated by negatively charged surfaces, plasma kallikrein and active Hageman factor. Tissue prokallikrein is activated by immunological reactions, plasma kallikrein, plasmin and autolysis. Tissue kallikrein, a single chain acidic glycoprotein, is more widely distributed in tissues: including kidney, pancreas, intestine, salivary glands, synovial tissue (Nustad *et al.*, 1975; Zeitlin & Smith 1973; Sharma *et al.*, 1983) and in polymorphonuclear leukocytes, particularly neutrophils (Figueroa *et al.*, 1989). Following activation, kallikreins act on kininogens to release the active kinin fragments; there are two main kininogens, HMW-kininogen and low molecular weight (LMW)-kininogen. Both are encoded by a single gene, but the HMW-kininogen has six domains whereas the LMW-kininogen has five (Muller-Esterl *et al.*, 1986). Plasma kallikrein acts on high molecular weight HMW-kininogen to cleave proteolytically releasing the nonapeptide bradykinin (Bk : Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg). Generally, tissue kallikrein acts on LMW-kininogen to cleave and release kallidin (KD : Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) apart from the mouse and rat where the enzyme releases Bk from LMW-kininogen. However, *in-vitro,* tissue kallikrein can release kinins from both HMW- and LMW-kininogen (Iwanaga *et al.*, 1977; Girolami *et al.*, 1986). In the rat however, tissue kallikrein does not release KD; Okamoto & Greenbaum (1983) discovered a rat specific LMW-kininogen (T-kininogen) which was resistant to kallikreins but released Ile-Ser-Bk (T-kinin) after hydrolysis with high concentrations of

trypsin. T-kininogenase, endopeptidase K and an acid proteinase all release T-kinin from T-kininogen (Xiong *et al.*, 1990; Gutman *et al.*, 1988; Sakamoto *et al.*, 1988).

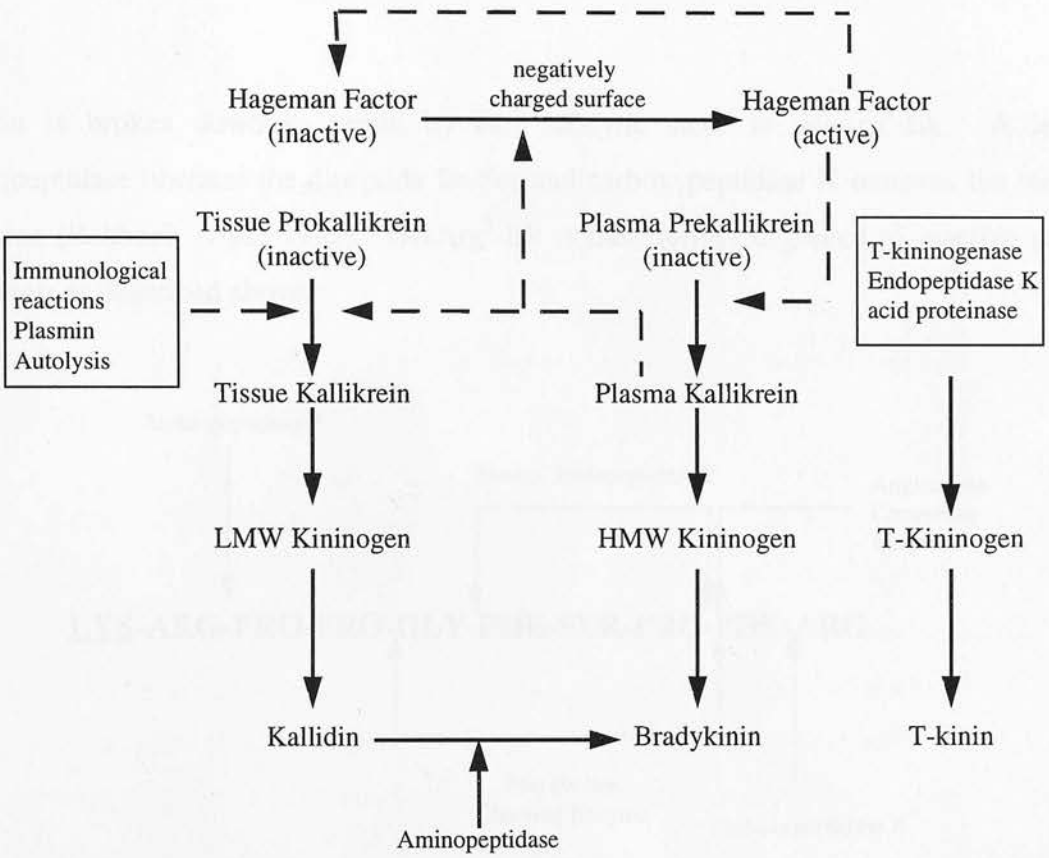


Figure 1.1

The kallikrein-kinin forming system.

Broken lines signify a substance which activates another, solid lines show substances which act on other substances to produce or release others.

1.3.1.2 Degradation

Kinins are often termed autacoids (local hormones) due to their localised action and rapid breakdown. Indeed, they are almost completely degraded (90 - 95%) after one pass through the pulmonary vascular bed (Ferreira & Vane, 1967). The half-life of Bk is approximately

one minute in plasma. There are two main kinin degrading enzymes (kininases), kininase I (carboxypeptidase N) which cleaves the terminal Arginine to form desArg^9-Bk or desArg^{10}-KD and kininase II (angiotensin converting enzyme) which liberates the Phe^8-Arg^9 dipeptide. Kinins can then be further degraded to liberate smaller inactive peptide fragments by neutral endopeptidase, aminopeptidase P and post proline cleaving enzyme as illustrated in Figure 1.2.

T-kinin is broken down in serum by two catalytic steps to desArg^9-Bk. A leucine aminopeptidase liberates the dipeptide Ile-Ser and carboxypeptidase N removes the terminal Arginine (Rehbock *et al.*, 1992). DesArg^9-Bk is then further degraded to inactive peptide fragments as described above.

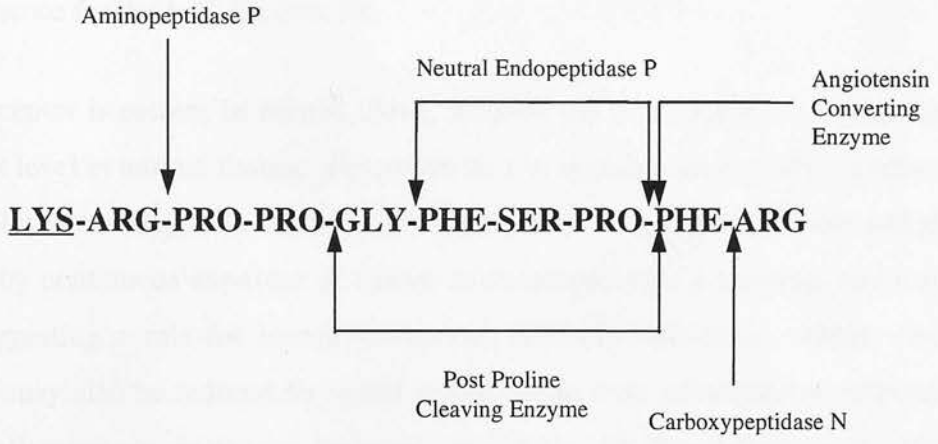


Figure 1.2

Breakdown of kinins.

The arrows show the site of action of degrading kinin enzymes for the breakdown of Bk and KD (underlined).

1.3.1.3 Receptors

Kinin receptors have been classified according to the relative potencies of agonists and antagonists. Two main classes of receptor were found by studies *in-vitro* on smooth muscle preparations from the rabbit, namely the aorta and the jugular vein. These have been termed kinin B_1 (Regoli *et al.*, 1977a) and B_2 receptors respectively (Regoli *et al.*, 1977b). Kinin B_2

receptors, although they differ slightly between species, generally exhibit the following rank order of potency (Regoli *et al.*, 1993):

$$KD > Bk > T\text{-kinin} > desArg^{10}\text{-}KD > desArg^{9}\text{-}Bk$$

Many of the effects of Bk, *in-vitro* and *in-vivo*, are mediated through interaction with the B_2 receptor. The kinin B_1 receptor generally exhibits a rank order of potency which is almost directly the inverse to that of the B_2 receptor (Regoli *et al.*, 1993):

$$desArg^{10}\text{-}KD > desArg^{9}\text{-}Bk > KD > Bk > T\text{-kinin}$$

In general terms, B_1 receptors show a much greater affinity for the kininase I metabolites of KD and Bk, $desArg^{10}$-KD and $desArg^{9}$-Bk. For example, the B_1 receptor exhibits a 10- to 50-fold preference for $desArg^{9}$-Bk over Bk.

The B_2 receptor is present in normal tissue, whereas the B_1 receptor is not expressed to any significant level in normal tissues. Responses to a B_1 agonist, $desArg^{9}$-Bk, increase over time in the rabbit aorta (Regoli *et al.*, 1977). This increase in B_1-mediated contraction could be inhibited by continuous exposure of tissues to dexamethasone a steroidal anti-inflammatory agent, suggesting a role for immunocompetent cells (Deblois *et al.*, 1988). B_1-mediated responses may also be induced by agents which produce an inflammatory response, such as LPS or inflammatory mediators, including interleukin 1β (Bouthillier *et al.*, 1987). This induction or up-regulation of receptors is probably due to *de novo* synthesis, as it can be blocked by the protein synthesis inhibitor, cyclohexamide (De Blois *et al.*, 1991).

Development of selective kinin receptor antagonists has furthered the understanding of the kinin receptor classes. Substitution of amino acid residues in the kinin structure led to the development of what are termed first generation antagonists. A selective kinin B_1 antagonist was developed by substituting a leucine amino acid residue at position 8 of the $desArg^{9}$-Bk peptide to give $desArg^{9}[leu^{8}]$-Bk. This antagonist was active in the rabbit aorta against $desArg^{9}$-Bk, but was inactive in the rabbit jugular vein against Bk (Regoli *et al.*, 1977). The first kinin B_2 antagonist, $[D\text{-}Phe^{7}]$-Bk, involved replacement of the proline residue at position 7 with the D-enantiomer of phenylalanine (Vavrek & Stewart, 1985). Incorporation of D-

phenylalanine at this position yields a peptide which is resistant to degradation by kininase II (Togo *et al.*, 1989). Extension of the peptide at the N-terminus by adding a D-Arginine residue prevents the action of aminopeptidase N (Stewart, 1992). Further amino acid residue substitutions have yielded antagonists with a greater affinity, which are more selective for the B_2 receptor. The best of these, with respect to affinity and selectivity, is D-Arg[Hyp^3,D-Phe^7,Leu^8]-Bk which has a pA_2 of almost 8.9 in the rabbit jugular vein compared to 5.8 in the rabbit aorta. Development of what are termed the second generation antagonists has increased the selectivity of antagonists for the B_2 receptor. The first of these was the Hoechst antagonist HOE 140, which has since been named icatibant. Hock *et al.* (1991) modified the D-phenylalanine at position 7 and the proline at position 8 to give a peptide which contained two synthetic amino acid residues and which was resistant to enzymatic degradation : D-Arg[Hyp^3,Thi^5,Tic^7,Oic^8]-Bk. This antagonist had a pA_2 in the rabbit jugular vein of 9.2 and was inactive on the rabbit aorta against desArg^9-Bk. Removal of the terminal Arginine converted icatibant into a selective B_1 receptor antagonist desArg^{10}-HOE 140 (Wirth *et al.*, 1991).

The apparent differences in potency of these antagonists in different tissues has led several groups to suggest that there may be several sub-types of the B_2 receptor. For example D-Arg[Hyp^3,D-Phe^7,Leu^8]-Bk has a pA_2 value of 8.9 in the rabbit jugular vein compared to 6.8 in the guinea pig ileum, which led to a proposal that different subtypes of the B_2 receptor were present in the different tissues: B_{2A} in the rabbit jugular vein and B_{2B} in the ileum. However, icatibant was shown to have an equally high affinity in both preparations suggesting that the difference may be species dependent (Regoli *et al.*, 1992).

Farmer *et al.* (1989) have suggested a third class of kinin receptor which they identified on guinea-pig tracheal smooth muscle. They found that two B_2 receptor antagonists, D-Arg[Hyp^3,D-Phe^7]-Bk and D-Arg[Hyp^3,D-Phe^7,Leu^8]-Bk, and the B_1 receptor antagonist, desArg^9[leu^8]-Bk, were unable to block Bk-induced contractions of tracheal smooth muscle. They suggested the presence of a new class of receptor which they called the B_3 receptor. However, this suggestion has been questioned after Hock *et al.* (1991) found that icatibant blocked the effect of Bk in the isolated guinea pig trachea. Icatibant contains strong hydrophobic residues that improve occupation of the receptor in different species (Regoli *et*

al., 1992).

Although the development of these second generation antagonists has led to discovery of increasingly more potent and biologically active antagonists, they are all peptides and thus subject to metabolism (with the exception of HOE 140) and poor bioavailability (Ward, 1990). The development of a non-peptide kinin antagonist was considered an important advance. Sawutz *et al.* (1994) discovered the first non-peptide potent antagonist, [[4-[[2-[[bis(cyclohexylamino)methylene]amino]-3-(2-(napthyl)-1oxopropyl]amino]phenyl]methyl] tributylphosphoniumchloride monohydrochloride (WIN 64338), which was selective for the B_2 receptor. More recently, Asano *et al.* (1997) identified an orally active non-peptide antagonist, which was selective for the kinin B_2 receptor. (E)-3-(6-acetamido-3-pyridyl)-N-[2-4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl] acrylamide (FR173657) inhibited Bk-induced bronchoconstriction in the guinea pig following oral administration, with an ED_{50} of $0.075 mgkg^{-1}$. FR173657 was also shown to be as potent as icatibant and was active at the human B_2 receptor.

Table 1.1. Summary table of pA_2 values of kinin antagonists in different smooth muscle preparations in-vitro.

compound	B_1 rabbit aorta	B_2 rabbit jugular vein	B_2 guinea pig ileum
[D-Phe^7]-Bk	5.94	-	-
D-Arg[Hyp^3,D-Phe^7,Leu^8]-Bk	5.76	8.9	6.77
Icatibant	inactive	9.17	8.94
WIN 64338	< 4	5.7	8.19
FR173657	-	-	9.2
desArg^9[Leu^8]-Bk	7.3	inactive	inactive
desArg^{10}-HOE 140	7.5	inactive	inactive

The two classes of receptor have been cloned; the rat B_2 receptor was cloned (McEachern *et al.*, 1991) and this was followed by the human B_2 receptor (Hess *et al.*, 1992). Menke *et al.* (1994) have since cloned the human B_1 receptor: the mRNA coding for the B_1 receptor which co-existed with the mRNA coding for the B_2 receptor, but was significantly shorter than the B_2 mRNA (Webb *et al.*, 1994). The B_1 receptor has 36% homology with the kinin B_2 receptor (Menke *et al.*, 1994). Although both receptors are encoded by different genes, both receptors have the seven transmembrane domain structure typical of G-protein coupled receptors. Between species the individual receptors are more homologous. At the amino acid level, the kinin B_2 receptors from rodents (rat and mouse) is 77% homologous to the human receptor (Brown *et al.*, 1995). For the B_1 receptor the homology between rat and human is 72% (Jones *et al.*, unpublished data).

1.3.1.4 Second messengers

Bk is unusual in the fact that when it binds to the B_2 receptor it can activate almost every second messenger system, depending on the location of the receptor. On sensory neurones when Bk binds to the B_2 receptor which is linked to a G-protein, it produces a depolarisation due to an increase in membrane permeability to Na^+ (Dunn & Rang, 1990; McGuirk & Dolphin, 1992). This influx of ions may activate a voltage dependent Ca^{++} channel and a subsequent influx of Ca^{++}. Bk also stimulates membrane bound phospholipase C and the subsequent generation of inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG). IP_3 can then elevate intracellular Ca^{++} by inducing it's release from intracellular stores (Burgess *et al.*, 1989; Dray & Perkins, 1993). DAG activates intracellular protein kinase C which in turn has been shown to inhibit membrane Ca^{2+} conductance (Dray *et al.*, 1988).

On non-neuronal cells, including fibroblasts, synovial cells and isolated smooth muscle cells, Bk acting through a B_2 receptor also activates membrane phospholipase A_2 resulting in the production of prostanoids (Bareis *et al.*, 1983; Burch & Axelrod, 1987; Bathon *et al.*, 1989; Galizzi *et al.*, 1994). Prostanoids, acting on prostanoid receptors, can activate adenylate cyclase and thereby raise levels of cAMP which in turn inhibits Ca^{++}-dependent K^+ permeability - which underlies a post-spike hyperpolarisation. This probably underlies the mechanism by which prostanoids can sensitise sensory neurones to Bk (Weinreich, 1986).

Apart from rapid enzymatic degradation, there are several other regulatory mechanisms involved in Bk activity. Bk releases nitric oxide (NO) from L-Arginine via B_2 receptors on vascular endothelial cells and subsequent activation of nitric oxide synthase. NO can then activate cellular guanylate cyclase to increase intra-cellular cGMP. cGMP then activates cGMP-dependent kinases which phosphorylate the kinin B_2 receptor, resulting in receptor desensitisation (McGehee *et al.*, 1992; Burgess *et al.*, 1989). Desensitisation of the receptor may also involve down regulation of receptor number or internalisation of receptor proteins (Roberts & Gullick, 1990).

Although distinctly different from the kinin B_2 receptor, many of the second messengers involved following B_1 receptor activation are the same. Activation of this receptor produces an increase in intracellular Ca^{++} in smooth muscle cells (Smith *et al.*, 1994; Marsh & Hill, 1994) and stimulation of membrane bound phospholipase C (Levesque *et al.*, 1993). As with the B_2 receptor, activation of the B_1 receptor activates membrane bound phospholipase A_2 with subsequent release of prostanoids from endothelial cells, smooth muscle cells, fibroblasts and macrophages (D'Orleans-Juste *et al.*, 1989; Galizzi *et al.*, 1994; Marceau & Tremblay, 1986; Tiffany & Burch, 1989; Marceau *et al.*, 1995).

1.3.1.5 Kinins in inflammation and nociception

1.3.1.5.1 Involvement of kinin B_2 receptors

Bk has all the properties of an inflammatory mediator. When injected into experimental animals and humans it produces inflammation (Marceau *et al.*, 1983), and levels of Bk have been shown to be elevated in acute inflammation and several chronic inflammatory diseases such as rheumatoid arthritis (Hargreaves *et al.*, 1988; Sharma & Buchanan, 1993). Injection of Bk evokes a vasodilation which is mediated both directly through B_2 receptors located on vascular smooth muscle cells and indirectly by an action on B_2 receptors on endothelial cells, smooth muscle cells and inflammatory cells to release nitric oxide and prostanoids (Bareis *et al.*, 1983; Burch & Axelrod, 1987). In inflammatory conditions, the rapid breakdown of Bk by kinases results in vasodilation localised to the site of inflammation. Bk when injected into the skin of animals and humans produces a localised oedema by acting on B_2 receptors on vascular endothelial cells, resulting in an increased vascular permeability and subsequent

leakage of plasma and inflammatory cells into the surrounding tissue (Marceau *et al.*, 1983). Bk also causes leukocyte accumulation to the site of tissue injury (Lewis, 1970), and binds to B_2 receptors on certain leukocytes to release further inflammatory mediators, including cytokines and prostanoids (Burch & Axelrod, 1987; Tiffany & Burch, 1989). This accumulation of leukocytes, some of which contain kallikreins and their substrates, kininogens (Figueroa *et al.*, 1992), at the site of tissue injury and inflammation further enhances the inflammatory process.

In inflammatory conditions there is usually an associated hyperalgesia. Bk when administered to the human blister base has been shown to be one of the most potent algesic agents known (Whalley *et al.*, 1987). B_2 receptors have been shown to be localised on sensory neurones in the dorsal root (Steranka *et al.*, 1988) and activation of these receptors has been shown to excite and sensitise peripheral C-fibres in the cat knee joint (Kanaka *et al.*, 1985). Activation of these peripheral sensory neurones can release sensory neuropeptides, including substance P and CGRP, and nitric oxide from sensory nerve terminals (Teixeira *et al.*, 1993). These sensory neuropeptides cause local vasodilation and an increase in vascular permeability, and thus further facilitate the inflammatory process. Furthermore, B_2 receptor antagonists are anti-nociceptive against the hyperalgesia which accompanies inflammation-induced by carrageenin (Costello & Hargreaves, 1989), formalin (Haley *et al.*, 1989), Freund's Complete Adjuvant, or UV irradiation (Perkins *et al.*, 1993) and peridontal inflammation (Griesbacher *et al.*, 1994).

1.3.1.5.2 Involvement of kinin B_1 receptors

Kinin B_1 receptors were originally thought to have a limited role. When B_1 receptor agonists were injected into naïve animals there was no inflammation or hyperalgesia produced (Davis & Perkins, 1994) and B_1 receptor antagonists were inactive at reversing Bk-induced inflammation (Marceau *et al.*, 1983). Indeed, evidence for B_1 receptors was only observed in vascular smooth muscle in particular the rabbit aorta where responses to desArg^9-Bk were seen to increase with time (Regoli *et al.*, 1977). However in recent years there has been increasing evidence for a role of the kinin B_1 receptor, particularly in inflammatory conditions and the accompanying hyperalgesia. B_1 receptors and responses can be induced by bacterial lipopolysaccharide and inflammatory mediators such as interleukin-1 β (Deblois *et al.*, 1991).

Kininase I activity is also increased in human inflammatory synovial fluid (Chercuitte *et al.*, 1987) and nasal discharge in allergic rhinitis (Proud *et al.*, 1987). This increased enzyme activity would result in an increased breakdown of kinins that are active at B_2 receptors to metabolites active at B_1 receptors. Following treatment of rats with lipopolysaccharide, B_1 receptor agonists were observed to induce an increase in paw oedema, secondary to an increase in vascular permeability (Campos *et al.*, 1996). Ahluwalia & Perretti (1996) found that following IL-1 β, desArg^9-Bk increased leukocyte accumulation in the mouse air pouch, an effect which was reduced by B_1, but not B_2 receptor antagonists. B_1 receptors on macrophages have been found to induce the release of inflammatory mediators, in particular cytokines (Tiffany & Burch, 1989), thus amplifying the inflammatory process.

The role of B_1 receptors in the hyperalgesia associated with inflammatory conditions has been of great interest in recent years. B_1 receptor agonists have been found to be nociceptive in a number of inflammatory pain models. Following injection of FCA into the knee B_1 agonists have been found to be hyperalgesic and antagonists have been found to be anti-nociceptive (Davis & Perkins, 1994; Perkins *et al.*, 1993); similar results have been found in UV-induced thermal hyperalgesia (Perkins & Kelly, 1993). In the formalin model of hyperalgesia both B_1 and B_2 receptor antagonists were reported to be anti-nociceptive (Correa & Calixto, 1993). IL-1 β when injected either into the rat paw or knee joint evokes a B_1-mediated mechanical hyperalgesia (Ferreira *et al.*, 1988; Davis & Perkins, 1994) or, when injected into the rat paw, a B_1-mediated thermal hyperalgesia (Perkins & Kelly, 1994). As yet there has been no direct evidence of B_1 receptors located on sensory neurones. In fact Davis *et al.* (1996) found no evidence of B_1 receptors on dorsal root ganglion cells removed from rats which had been pre-treated with Freunds complete adjuvant. It therefore seems more likely that B_1 receptors are induced, or upregulated on non-neuronal cells, such as macrophages, fibroblasts, synovial cells, endothelial cells and smooth muscle cells (Tiffany & Burch, 1989; Lerner & Modeer, 1991; Bathon *et al.*, 1989; Cahill *et al.*, 1988; Galizzi *et al.*, 1994). B_1 receptor agonists may then act on these cell types to release other pro-inflammatory or algesic agents to potentiate the inflammatory response.

1.3.2 Cytokines

Cytokines are a family of soluble proteins or glycoproteins which are produced by leukocytes and many other cell types, and generally act as chemical communicators between cells (Rothwell & Hopkins, 1995). The cytokine family comprises a range of mediators including: interleukins (IL-1α, IL-1β, IL-2 - IL-14), growth factors, interferons and haematopoietic factors. Once released from cells they act on specific receptors on target cells to produce a host of actions and interactions with each other, often termed 'the cytokine network', and other inflammatory mediators, including kinins and prostanoids. To cover all the individual actions, interactions and modulations occurring within the cytokine network is outwith the scope of this thesis which will concentrate mainly on IL-1β and two anti-inflammatory, or modulatory cytokines IL-4 and IL-13.

1.3.3 Interleukin-1

1.3.3.1 Formation

IL-1 consists of two distinct but related glycoproteins, IL-1α and IL-1β, which are encoded by separate genes and in most human tissues IL-1β mRNA predominates (Arai *et al.*, 1990). Interestingly the gene which encodes IL-1β is not spontaneously expressed but can be stimulated by LPS, complement components or IL-1β itself to increase production of IL-1β mRNA rapidly. IL-1α and IL-1β are synthesised as 31kD precursors (pro-IL-1) and converted to 17.5kD active glycoproteins (Dinarello *et al.*, 1994). Pro-IL-1α can be found on the surface of cells in a form which is biologically active (Kurt-Jones *et al.*, 1985), whereas pro-IL-1β requires cleavage for it's optimal secretion and biological activity (Fenton *et al.*, 1988; Dinarello, 1994). The enzyme responsible for this is Interleukin converting enzyme (ICE) which itself is present in numerous cell types as an inactive precursor (Thornberry *et al.*, 1992; Cerretti *et al.*, 1992). In humans IL-1α is 26% homologous to its IL-1β relative (Priestle *et al.*, 1989; Graves *et al.*, 1990). IL-1 can be produced and released by a variety of cells including, macrophages, fibroblasts, keratinocytes, T- and B-lymphocytes, astrocytes, microglia, endothelial cells, smooth muscle cells and chondocytes (Callard & Gearing, 1994).

1.3.3.2 Receptors

There are two main members of the IL-1 receptor family, IL-1RI and IL-1RII both of which are encoded by a common receptor gene (Sims *et al.*, 1995). Both receptors have a single transmembrane domain, but whereas the IL-1RI has a long cytosolic domain (213 amino acids) (Sims *et al.*, 1988) the cytosolic domain of the IL-1RII is relatively short (29 amino acids) (McMahan *et al.*, 1991). It is not surprising then, that this receptor doesn't transduce any cell-signalling pathways (Sims *et al.*, 1993).

IL-1RI is constitutively expressed in low numbers on nearly all cell types, however the number of cell surface receptors increases rapidly on exposure to a number of inflammatory mediators including; IFNγ, IL-1, IL-2, IL-4, PGE_2, PDGF, phorbol esters and promoters of inflammation, e.g. LPS (Akahoshi *et al.*, 1988; Koch *et al.*, 1992). IL-1RII is found primarily on the surface of neutrophils, monocytes and B-lymphocytes (McMahan *et al.*, 1991) and like the IL-1RI the number of receptors present is also increased during inflammation by a variety of mediators including; corticosteroids, IL-1, PGE_2 and IL-4 (Re *et al.*, 1994; Colotta *et al.*, 1995; Colotta *et al.*, 1993).

IL-1RII can be shed from the cell surface and is often found as a soluble receptor in the circulation (Symons *et al.*, 1990). There is increasing evidence that this receptor acts as a "decoy receptor": it binds preferentially and irreversibly to IL-1β, thus mopping up excess IL-1β produced in inflammation, controlling the biological actions of IL-1β (Colotta *et al.*, 1994). This would seem to be a powerful modulatory role, as normal levels of the soluble receptor are approximately 100 pmoles, whereas even following infection IL-1β levels only rise to 25 pmoles (Dinarello *et al.*, 1994).

1.3.3.3 Second messengers

The levels of IL-1 required to produce its biological action are very low; concentrations in the low picomolar range produce effects. Indeed, it seems that only 2 to 3% of Il-1RI need to be occupied for a biological action to occur. This suggests that IL-1 receptor signal transduction and amplification are highly efficient. However, the exact mechanism underlying this is still

not fully understood and some aspects of signal transduction following receptor binding remain unclear (O'Neill, 1995). It would seem that IL-1 triggers a diverse range of second messengers, receptor activation increases levels of cAMP via a G-protein (Chedid *et al.*, 1989; O'Neill *et al.*, 1997). The mechanism by which the receptor couples to the G-protein is unconventional as the receptor doesn't have the usual seven transmembrane domains typically observed in G-protein coupled receptors (Dohlman *et al.*, 1987). Receptor activation also induces a hydrolysis of phospholipids by non-phosphatidylinositol phospholipase C (Rosoff *et al.*, 1988; Kester *et al.*, 1989), release of ceramide from sphingomyelin (Mathias *et al.*, 1993) and release of arachidonic acid following activation of phospholipase A_2 (Gronich *et al.*, 1994).

Phosphorylation of proteins follows receptor activation, and is usually accomplished by protein kinases (PK). IL-1 is not different in this respect except it activates a whole range of PK's including PKC and PKA (Munoz *et al.*, 1990; Mizel, 1990). In addition IL-1 also activates mitogen-activated protein (MAP) kinase (Guy *et al.*, 1991). Indeed, it now seems that IL-1 initiates a protein kinase cascade involving MAP kinase and kinases, which may be the manner in which the IL-1 signal is amplified and it's biological actions seemingly much greater than receptor occupancy (O'Neill *et al.*, 1995).

1.3.3.4 Inhibitory modulation

Modulation or inhibition of the effects of IL-1β can be accomplished in a number of ways, ranging from reducing the amount of IL-1β released, antagonism of IL-1 at a receptor level to modulation of its actions by anti-inflammatory cytokines. Inhibition of the enzyme responsible for cleavage of pro- IL-1β to it's active form would reduce levels of IL-1β released from cells and thus levels of IL-1β in the circulation. Mice deficient of ICE have been shown to be unable to produce the mature form of IL-1β (Li *et al.*, 1995). This finding led to the study and development of specific inhibitors of ICE. Several inhibitors of ICE have been found and their effectiveness at reducing levels of IL-1β has been studied.

An endogenously released inhibitor of IL-1 activity was detected in the serum of humans injected with LPS (Dinarello *et al.*, 1981) and in the urine of patients with monocytic

leukaemia (Seckinger and Dayer, 1987). The latter of these has been the most studied and characterised as an IL-1 inhibitor and has been termed IL-1 receptor antagonist (IL-1ra) (Seckinger *et al.*, 1990). IL-1ra is more closely related to IL-1β (26% homology) than IL-1α (18% homology) and all have a similar three-dimensional structure (Vigers *et al.*, 1994; Priestle *et al.*, 1989; Graves *et al.*, 1990). It is therefore not surprising that they all bind to the same receptor, however, IL-1ra lacks a specific binding site for the accessory protein, rendering it able to bind to the receptor but unable to transduce a signal (Evans & Robbins, 1994). Thus, IL-1ra competes with IL-1β for it's receptor and acts as a competitive antagonist. IL-1ra reduces the severity of a number of inflammatory diseases including haemodynamic shock induced by LPS in rabbits (Fischer *et al.*, 1992a); adjuvant-induced arthritis in rats (Schwab *et al.*, 1991); inflammatory bowel disease in rabbits (Cominelli *et al.*, 1990) and neutrophil accumulation in inflammatory peritonitis (McIntyre *et al.*, 1991). Levels of IL-1ra have been observed to increase in inflammatory diseases in experimental animals and humans (Fischer *et al.*, 1992b) and therefore may act to regulate and control the biological effects of IL-1. A tripeptide antagonist (Lys-D-Pro-Thr) has also been developed which selectively acts on the IL-1 receptor and has been used to regulate the biological effects of IL-1 (Ferreira *et al.*, 1988).

Although cytokines are mainly thought of as pro-inflammatory molecules, there are several which have been found to have anti-inflammatory properties (Burger & Dayer, 1995) and are involved in regulating or modulating the biological actions of inflammatory cytokines in particular IL-1β. There are three such cytokines; IL-4, IL-10 and IL-13. The manner in which these modulate the effects of IL-1β is multifarious.

IL-4, originally identified as a B-cell growth factor, is mainly produced by T_H2 cells (MacNeil *et al.*, 1990) and was subsequently found to be involved in proliferation, differentiation and activation of various cell types. However, it was also found to have the ability to suppress many of the pro-inflammatory effects of monocytes; IL-4 downregulates the production of IL-1 by monocytes (Essner *et al.*, 1989; te Velde *et al.*, 1990). In addition IL-4 upregulates the production of IL-1ra (Vannier *et al.*, 1992) and increases IL-1RII, the 'the decoy receptor', (Colotta *et al.*, 1993) thus further reducing the biological actions of IL-1.

IL-10, like IL-4, is produced by T_H2 cells (MacNeil *et al.*, 1990) but, also by monocytes activated by other cell types (Spits *et al.*, 1992) including monocytes activated by LPS (de Waal Malefyt *et al.*, 1991). The regulation of IL-1's biological actions by IL-10 are somewhat similar to those of IL-4; including downregulation of IL-1 production and upregulation of IL-1ra production (Jenkins *et al.*, 1994).

IL-13 is also secreted by activated T cells and mast cells (Burd *et al.*, 1995) and acts on a receptor which bears similarities to the receptor for IL-4 (Vita *et al.*, 1995; Smerz-Bertling & Duschl, 1995). It is not surprising, then, that IL-13 has similar regulatory actions to IL-4. IL-13 inhibits the production of inflammatory cytokines, including IL-1β, by monocytes activated by LPS (Minty *et al.*, 1993). IL-13 also increases expression and release of IL-1ra (Vannier *et al.*, 1996) and IL-1RII from monocytes and neutrophils (Colotta *et al.*, 1996; Girard *et al.*, 1996), thus further regulating the biological activity of IL-1.

1.3.3.5 Role in inflammatory pain

Due to its multifunctional actions IL-1β is one of the primary inflammatory mediators. A plethora of stimuli are known to induce IL-1 production including LPS, immune complexes, activators of macrophages, inflammatory mediators (including TNFα) (Epstein & Ramila, 1986; Billingham, 1987). Levels of IL-1 have been shown to be increased in inflammatory diseases.

The role of IL-1 in nociception has been extensively studied. Local injection of IL-1β has been shown to induce both mechanical (Ferreira *et al.*, 1988; Watkins *et al.*, 1994; Davis & Perkins, 1994) and thermal hyperalgesia (Perkins & Kelly, 1994). The hyperalgesia was interesting, in that local injection could induce a contralateral hyperalgesia, suggesting a systemic sensitisation of nociceptors at a site distal to the injection site (Ferreira *et al.*, 1988; Perkins & Kelly, 1994). These hyperalgesic actions can be antagonised by IL-1ra and the tripeptide, Lys-D-Pro-Thr, indicating that these effect are mediated via interaction with the IL-1 receptor (Ferreira *et al.*, 1988; Davis & Perkins, 1994). Whether these actions are mediated through interaction with IL-1 receptors located on sensory nerves is still unclear. IL-1β acts on rat dorsal root ganglion cells to produce an immediate increase in Ca^{++} suggesting that it

can act directly on sensory nerves (Kawatani & Birder, 1992). IL-1β has also been shown to increase spontaneous neural discharge when injected into the paw and also sensitises noxious cold- and heat-induced firing of sensory nerves (Fukuoka *et al.*, 1994). However, this increase in discharge is not instantaneous suggesting that it may not be due to direct activation of sensory nerves. It seems more likely that the effects of IL-1β on sensory nerves is due to the release of other mediators which then act on the sensory nerves. IL-1 can induce prostaglandin release from a variety of cells, including macrophages, endothelial cells, fibroblasts and synovial cells (Billingham, 1987) and also upregulates the inducible cyclooxygenase enzyme (COX-2) in human synovial cells (Bathon *et al.*, 1994). However the role of prostaglandins in mediating IL-1β-induced hyperalgesia is not fully clear. Indomethacin has been shown to block the hyperalgesic effects of IL-1β in some studies (Cunha *et al.*, 1992; Davis & Perkins, 1994), but in others studies a prostaglandin-independent is evident (Follenfant *et al.*, 1989). Other possible candidates for mediating this hyperalgesia are kinins. IL-1β upregulates B_1 and B_2 receptors and enhances responses to kinins (Galizzi *et al.*, 1994; Lerner & Modeer, 1991). Indeed, the hyperalgesia produced can be blocked by kinin B_1 and B_2 antagonists (Davis & Perkins, 1994). Thus, IL-1β-induced hyperalgesia may be due to the release of other mediators which can act directly on sensory nerves.

This thesis studied the hypothesis that kinin B_1 and B_2 receptors are involved in mediating the hyperalgesia associated with inflammation and investigated the involvement of other inflammatory mediators (including, IL-1β, IL-4, IL-10, prostanoids and nitric oxide) in the modulation of kinin-mediated nociception.

2. MATERIALS AND METHODS.

2.1 Electrophysiological studies.

All experimental animals were housed in an animal house in cages (up to 5 per cage). Rooms (12h light/dark) were maintained at 20 °C and had food and water *ad libitum*. All experiments complied with Home Office regulations under project licence (PPL 60/1860) and personal licence (PIL 70/10043).

2.1.1 Induction of arthritis.

Male Wistar rats (250-300 g) were injected subdermally with 0.1ml of Freund's complete adjuvant (1.0 $mgml^{-1}$ heat killed *Myobacterium tuberculosis* in paraffin oil, Sigma) at two sites around the ankle joint under temporary anaesthesia induced by halothane (2% in oxygen). Experiments were performed on animals after at least 14 days during which time a localised swelling, redness and hyperalgesia of the injected ankle had developed and was maintained. The circumference of both ankles was measured using a tape measure. For knee joint experiments 0.1 ml of Freund's complete adjuvant was injected intra-articularly into the knee joint under halothane anaesthesia. The diameter of both knee joints was measured using calipers at the time of electrophysiological experiments.

2.2 Electrophysiological recordings.

2.2.1 Surgical procedures.

Male Wistar rats, weighing between 300 and 500 g were anaesthetised with urethane (ethyl carbamate 25% w/v, 0.6 $ml100g^{-1}$ body weight. i.p.). Body temperature was monitored and maintained at 37 ± 0.5 °C by a heating blanket connected to a thermistor probe inserted in the rectum. A midline incision was made in the ventral aspect of the neck, the trachea was cannulated and mean arterial blood pressure was monitored via a cannulated carotid artery. A further cannula was inserted into the right femoral artery and advanced until the tip was at the level of iliac bifurcation; this cannula was used for the close-arterial injection of drugs to the

left hind limb(intra-arterially: i.a.). Drugs were administered in a volume of 0.1 ml and washed in with 0.2 ml saline (0.9% w/v aqueous NaCl).

To record neural discharge from articular afferents innervating the ankle joint, the tibial nerve of the left hind limb was exposed by making a medial incision in the skin and then removing the muscles overlying the nerve. The primary articular-cutaneous ramus was identified where it left the medial plantar nerve (Guilbaud et al, 1985). This dissection provided sufficient skin flap to form a liquid paraffin pool (37 °C) covering the exposed nerve and ruptured of the cutaneous branches of the PACR. Neural recordings were thus restricted to activity originating in the joint capsule and surrounding tissues.

To record neural discharge from articular afferents innervating the knee joint, the saphenous nerve of the left hind limb was exposed by making a making a medial incision in the skin. A medial articular nerve was identified where it branched from the saphenous nerve and dissected from the muscle and connective tissue. Again, the dissection provided sufficient skin flap to form a liquid paraffin pool covering the exposed nerve and the cutaneous branches of the medial articular nerve were ruptured. Neural recordings were thus restricted to activity in nerves innervating the joint capsule and surrounding tissues (Figure 2.1).

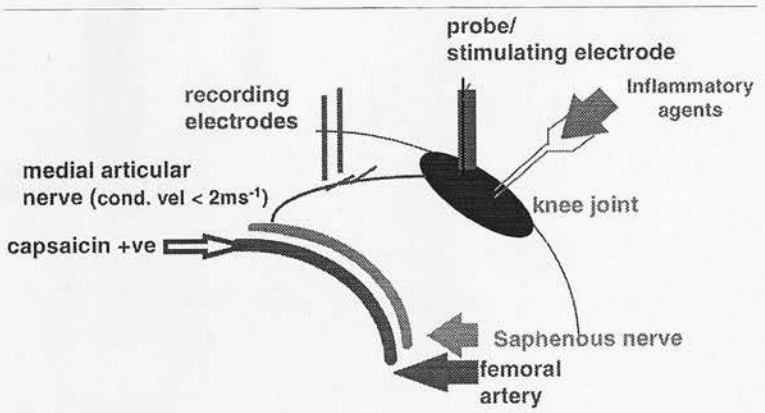


Figure 2.1

Schematic diagram of the dissection and location of the medial articular nerve innervating the rat knee joint.

2.2.2 Recording afferent neural activity.

Electrical activity of the PACR or MAN was recorded from fine nerve filaments and in both cases the nerve was cut centrally to abolish efferent nerve activity. Fluid (lymph), which accumulated close to the nerve, was removed by suction using a syringe connected to a fine nylon catheter to prevent short circuits. Electrical activity was recorded extracellularly using bipolar platinum-iridium electrodes. The electrical signal was amplified (Neurolog) and displayed on a storage oscilloscope (Tektronix 5113). The signal was then digitised using a digital audio processor (Sony PCM 701-ES) for storage on videotape (videotape recorder: Sony Betamax SL-HF100 UB). The output from the amplifier was passed through a voltage discriminator (Digitimer D130) so that action potentials of a selected amplitude could be counted. Figure 2.2. shows the arrangement used for recording and storing data.

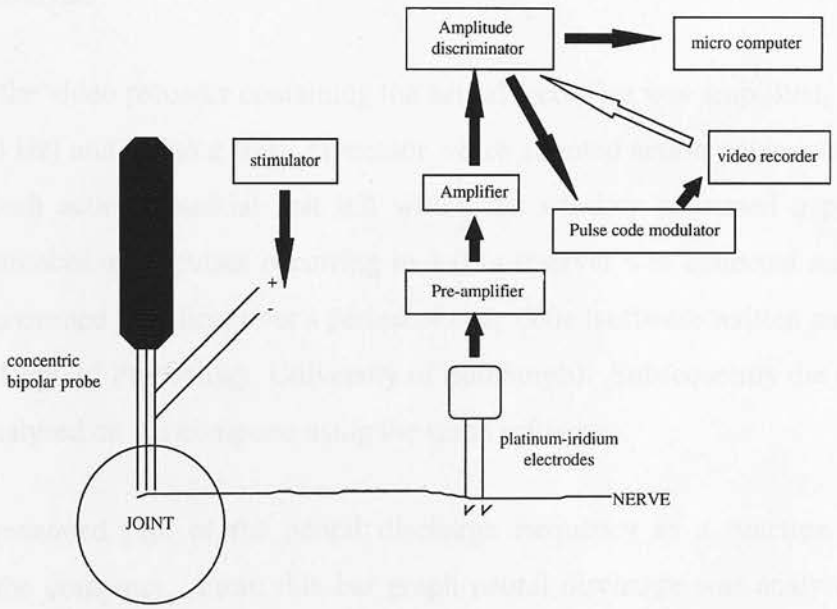


Figure 2.2

Schematic diagram showing the arrangement used for electrophysiological recording, collection and storage of data, and electrical stimulation of the nerve at the receptive field.

High threshold mechanonociceptors with receptive fields in the capsular tissue of either the

ankle or knee joint were identified by their activation in response to an intense mechanical stimulation with a smooth tipped plastic probe (0.5-1.0 mm diameter). A probe, consisting of a silver wire isolated from a metal cylinder casing was used to estimate the conduction velocity of the nerve. This bipolar arrangement gave a localised electrical stimulus of 1 ms pulse duration at 1 Hz; voltage was slowly increased (0.1 - 5 V) until spike activation occurred. Conduction velocity (v) was calculated by measuring the distance (d) between the stimulating electrode and recording electrodes and measuring the time (t) for the action potential to travel this distance ($v = d / t$, ms^{-1}). The distance measured between the stimulating and recording electrodes is an estimation of the length of nerve that the action potential travels along and assumes the nerve is relatively straight. The conduction velocity was therefore an estimate, but the identification of nociceptive fibres was always confirmed by testing the unit's sensitivity to capsaicin. Fibres were rejected which were not activated by intense mechanical stimulus or capsaicin. Successful recordings were achieved in approximately 60 - 70% of all experiments.

2.2.3 Data Analysis

The output of the video recorder containing the neural recording was amplified, filtered (band pass 10 - 1000 Hz) and fed to a spike processor which counted action potentials of a selected amplitude. Each action potential that fell within the window generated a pulse of 1 ms duration; the number of impulses occurring in a 0.1s interval was collected and stored on a computer programmed to collect over a period of over 600s (software written and supplied by Dr. M. Dutia, Dept. of Physiology, University of Edinburgh). Subsequently the data collected on disk was analysed on the computer using the same software.

A computer-generated plot of the neural discharge frequency as a function of time was displayed on the computer. From this bar graph neural discharge was analysed in defined intervals (pre-injection) and during defined intervals following injection of the drug. Drug effects were analysed in one of two ways:

A: the peak increase in neural discharge (measured over a 5 or 15s period) above the neural discharge in the pre-injection control period (measured over a 30s period).

B: the total number of impulses generated within the duration of action of the drug above the total number generated within the pre-injection control period (30s).

For example:

Δ (increase in discharge) = impulses (response period) - [impulses (control period,30s) * (duration of drug action/30)]

Analysis A only takes into account the maximum magnitude of the response whereas analysis B takes into account the duration of action of the drug.

2.2.4 Statistical analysis

Statistical analysis of differences between means was carried out using the non-parametric statistical tests. The Mann-Whitney U test and Wilcoxan matched pairs test were employed where appropriate, and the Null hypothesis rejected at $P < 0.05$ (two-tailed).

2.3 Behavioural studies

In all experiments male Wistar rats were used (90 - 100 g kept at 21 ± 2 °C , 12 h light / dark, food and water *ad libitum*). All experiments complied with Home Office regulations under project license (PPL 70/02111) and personal license (PIL 70/10043)

2.3.1 Mechanical hyperalgesia

The assessment of mechanical hyperalgesia involved the injection (100 µl, needle: 26 gauge, 3/8 inch) of inflammatory or hyperalgesic agents into the right knee joint under light anaesthesia (enflurane). Both hind paws were placed on separate balanced force transducers, amplified (Harvard transducer amplifiers 50-7996), digitised (CED 101plus) and the data collected on computer. A downward force was applied such that the non-injected knee tolerated 100 g, which could be visualised on the computer monitor. When this occurred the load tolerated by the injected leg was captured on computer (software written by T. Mulcahy, SIMR, London). Three control readings were taken before intra-articular injection of drugs. A time course of the drug effects was then carried out (0.5, 1, 2, 3 and 5h post-injection).

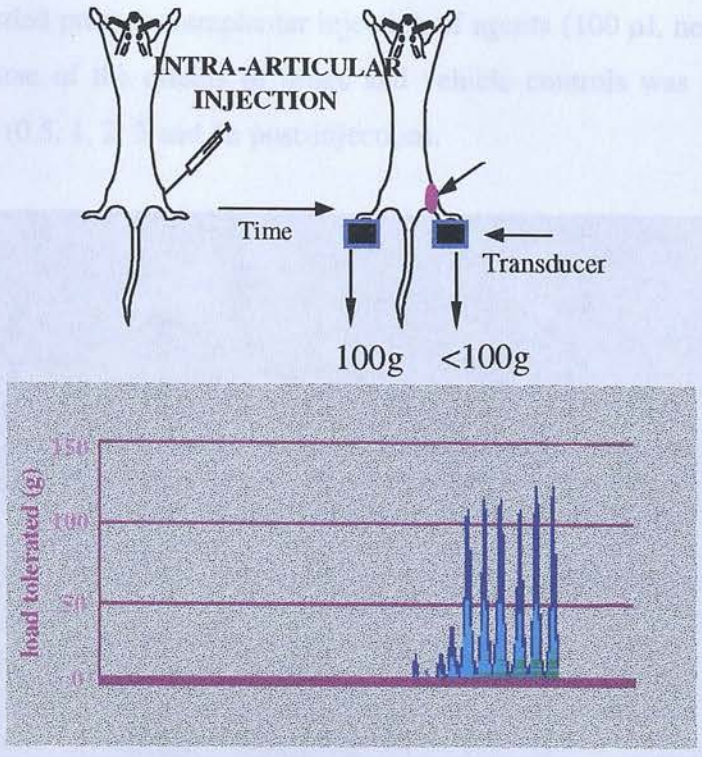


Figure 2.3

Diagram showing the apparatus used for assessing the mechanical hyperalgesia resulting from intra-articular injection into the right knee joint of the rat.

Bottom panel shows a screen capture from an experiment. The peaks at the rear show the load tolerated by the uninjected knee, the peaks in the foreground show the load tolerated by the injected knee.

2.3.2 Thermal hyperalgesia

The method for assessment of thermal hyperalgesia involved the injection of inflammatory or hyperalgesic agents into the plantar region of the left hind paw. Animals were the placed in separate compartments in a glass floored perspex box and allowed to acclimatise (10-20 min). The heat source was positioned manually directly beneath the paw. A ramp heat stimulus was applied, using a focused radiant heat beam, to the plantar surface of the paw and the latency to paw withdrawal measured (Ugo Basile, Plantar Test, (Hargreaves et al., 1988)). The hind paws were exposed to the radiant heat stimulus in a random manner so that no rat had both paws exposed immediately after each other. When the rat withdrew its paw, the sudden drop of reflected radiation switched off the heat source and stopped the time counter. Two control

readings were recorded prior to intraplantar injection of agents (100 µl, needle: 26 gauge, 5/8 inch). A time course of the effects of drugs and vehicle controls was then carried out at various time points (0.5, 1, 2, 3 and 5h post-injection).

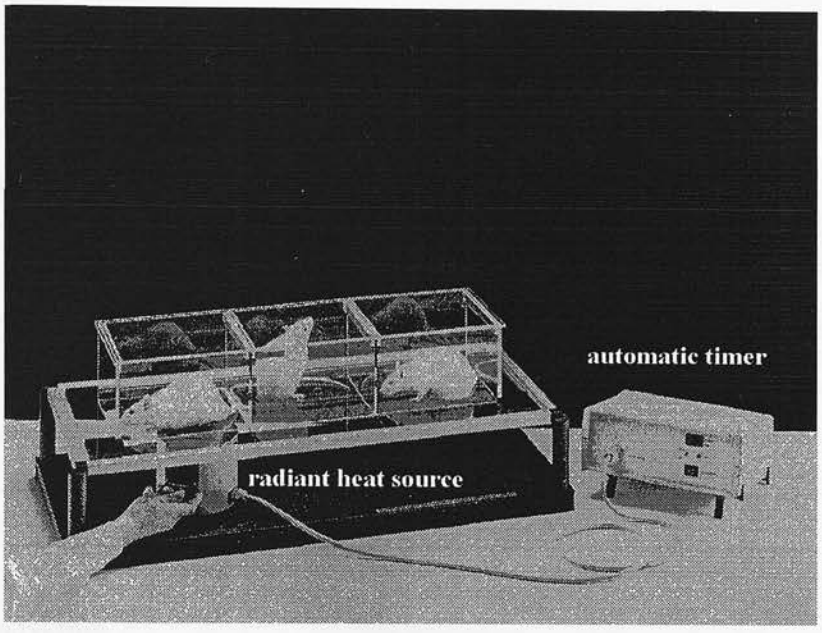


Figure 2.4

Diagram showing the apparatus used for assessing the thermal hyperalgesia resulting from intra-plantar injection into the left hind paw of the rat.

Figure shows radiant heat source directed at the plantar surface of the rat paw and the automatic timer which will recorded the latency when the paw was withdrawn.

2.3.3 Data analysis

In mechanical hyperalgesia experiments, when the load tolerated by the non-injected knee reached 100 g the load tolerated by the injected knee was captured by a computer. The load tolerated by the injected knee following drug administration was expressed as a percentage of the mean of the three pre-injection control measurements. Similarly thermal hyperalgesia data following intra-plantar injection was expressed as a percentage of the pre-injection control withdrawal latency.

(post-injection reading/mean control readings)*100

2.3.4 Statistical Analysis

In both behavioural tests the drug effects were compared to the pre-injection control readings using ANOVA followed by Honest Tukey's post-hoc test and the Null hypothesis rejected when $P < 0.05$.

2.4 Drugs

All drugs and source of drugs used in this thesis are listed below with the relevant solvents.

DRUG	SOURCE	molecular weight	SOLVENT
bradykinin	synthesised in house/Sigma	1261.7 / 1060.2	saline
capsaicin	synthesised in house	308	DMSO
D-Arginine	Sigma	210.7	saline
D-NAME	Sigma	269.7	saline
desArg^9-bradykinin	synthesised in house/Sigma	1039.2 / 904.0	saline
desArg^{9}leu^8-bradykinin	synthesised in house/Sigma	956.1 / 870.0	saline
Freund's complete adjuvant	Sigma	1mgml^{-1}	-
icatibant	synthesised in house	1694.3	saline
indomethacin	Sigma	357.8	2%$NaHCO_3$
interleukin-10	R&D Systems		saline
interleukin-1β	NIBSC		saline
interleukin-4	R&D Systems		saline
intreleukin-1ra	NIBSC		saline
L-Arginine	Sigma	210.7	saline
L-NAME	Sigma	269.7	saline
Urethane (ethylcarbamate)	Sigma	89.09	saline

3. THE ROLE OF NITRIC OXIDE IN THE MODULATION OF NEURAL DISCHARGE AND BK-INDUCED INCREASE IN C-FIBRES INNERVATING NORMAL AND ARTHRITIC RAT ANKLE JOINT.

3.1 Introduction.

Nitric oxide (NO) is a mediator which can be released from both neuronal and non-neuronal cells and may act as a messenger in both the central and peripheral nervous systems (Bredt *et al.*, 1990). Nitric oxide synthase (NOS) catalyses the hydroxylation/oxidation reaction which converts L-Arginine to citrulline and NO. There are three types of NOS a constitutive enzyme, an inducible enzyme, which can be induced by inflammatory mediators, and a neuronal form. Nitric oxide interacts with the haem group of soluble guanylate cyclase to increase levels of guanosine 3':5-cyclic monophosphate (cyclic GMP) [see Moncada *et al.*, 1991 for review] and can directly activate calcium-dependent potassium channels (Bolotina *et al.*, 1994).

A modulatory role for the L-Arginine-NO-cyclic GMP pathway in peripheral and central nociceptive processing has been suggested. In the periphery, the NO-cGMP pathway has been shown to be involved in both hyperalgesia and antinociception. For example, in the formalin model in the rat, spinal neural activity is reduced by concomitant administration (with formalin) of L-NAME, a NOS inhibitor, into the receptive field (Haley *et al.*, 1992). However topical administration of nitroglycerin, which can raise levels of NO is antinociceptive in the Randall-Selitto paw pressure test (Ferreira *et al*, 1991). In addition, L-Arginine, a NO precursor, is antinociceptive in carageenan-induced mechanical hyperalgesia (Duarte *et al.*, 1992) and, depending on the dose used, has been reported to be both hyperalgesic (0.1 - 1μg, intraplantar) and antinociceptive (10 μg, intraplantar) in the formalin-induced paw licking model of hyperalgesia (Kawabata *et al.*, 1993).

Bradykinin has excitatory and sensitising actions on sensory nerves (Dray *et al.*, 1988; Rang *et al.*, 1991), and produces pain when applied to the human nasal mucosa, or to a blister base (Whalley *et al.*, 1987). Kinin levels have been shown to be elevated in synovial fluids from patients with inflammatory conditions such as rheumatoid arthritis (Jasani *et al.*, 1969) and

this raises the possibility that bradykinin plays a role in the hyperalgesia associated with these conditions. Bradykinin also causes release of nitric oxide from vascular endothelial cells (Bogle *et al.*, 1991), and a role for the NO-cGMP pathway in the desensitisation of bradykinin receptors has been suggested. Sodium nitroprusside, a NO donor, increased bradykinin desensitisation whereas NOS inhibitors reduced desensitisation in cultured sensory neurons (McGehee *et al.*, 1992), and responses of peripheral sensory nerves to bradykinin have been shown to be reduced by sodium nitroprusside and dibutyrl-cGMP (Dray *et al.*, 1992).

We have investigated the effects of a NOS inhibitor, L-NAME, and the NO precursor, L-Arginine, on the spontaneous neural discharge of articular high threshold mechanonociceptors recorded from normal and chronically inflamed (adjuvant-arthritic) ankle joints in rats anaesthetised with urethane. We have also determined whether responsiveness of the joint mechanononociceptors to bradykinin was affected by modulating the level of endogenous NO by using L-NAME and L-Arginine.

3.2 Methods.

As described in section 2.3.

3.3 Results.

Afferent units from fifteen normal and seventeen arthritic rat ankle joints were studied. For electophysiological experiments arthritic rats were used between 14 and 33 days (average 20 ± 1 days) after injection of adjuvant when the left ankle had swollen by 38 ± 2% (range: 21 - 48%) compared to the contralateral joint. All the units examined were excited by capsaicin (1 - 10 µg, i.a.) and the mean afferent conduction velocity was 0.67 ± 0.01 ms^{-1} (n = 17; range: 0.16 - 1.5 ms^{-1}) from normal and 0.64 ± 0.07 ms^{-1} (n = 17; range: 0.2 - 1.6 ms^{-1}) from arthritic ankle joints, i.e. within the range for C-fibre afferents (< 2.5 ms^{-1}).

Effects of L-NAME on the neural discharge of C-fibres.

L-NAME (10 - 20 mg.kg, i.a.), but not D-NAME (30 $mgkg^{-1}$, i.a.), increased neural discharge of C-fibre mechanonociceptor afferents (see Figure 3.1) in 82% (14/17 units from 9 experiments) of units from normal ankle joints and 82% (14/17 units from 8 experiments) of

units from arthritic ankle joints.

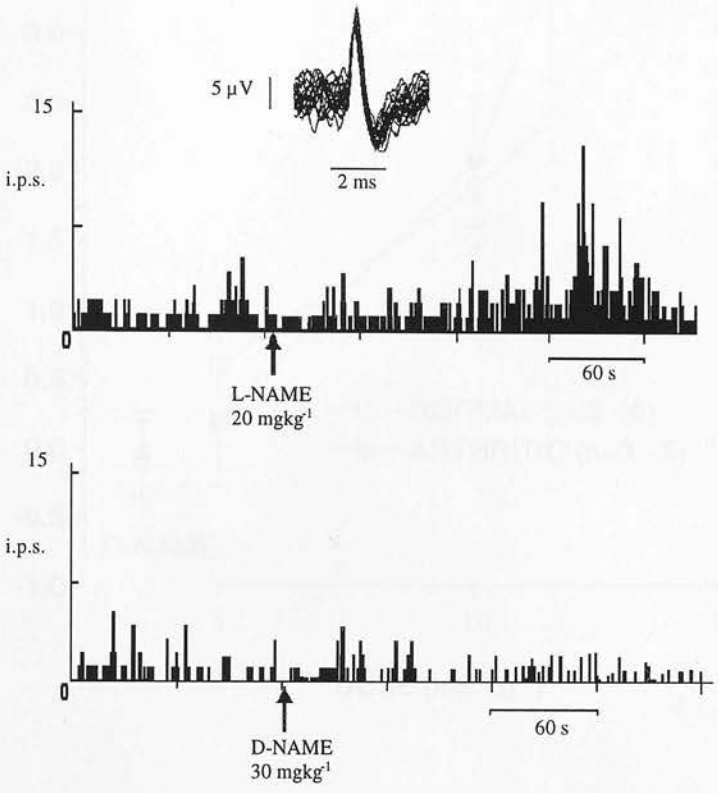


Figure 3.1

L-N^G nitroArginine methyl ester (L-NAME) but, not its inactive isomer D-N^G nitroArginine methyl ester (D-NAME) increases neural discharge from articular nociceptors.

Bargraphs illustrate the increase in discharge evoked by L-NAME (20 mgkg^{-1}, i.a. at arrow) [A] but not its inactive isomer D-NAME (30 mgkg^{-1}, i.a. at arrow)[B]. The inset illustrates 20 fast oscilloscope sweeps of the activated unit.

There was no difference in the increase evoked by L-NAME in afferents from normal or arthritic ankle joints, but in both cases it was significantly greater than that evoked by the inactive isomer, D-NAME (Figure 3.2) which had no effect on spontaneous neural discharge.

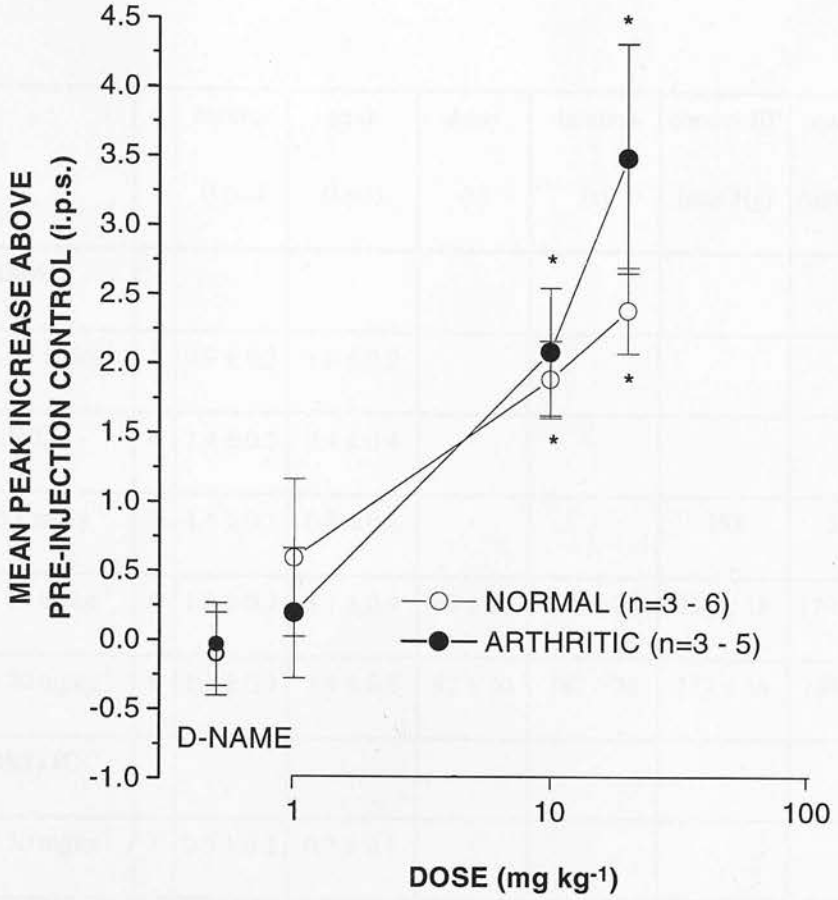


Figure 3.2

L-NAME increases neural discharge of articular nociceptors in normal and arthritic rat ankle joints.

The mean peak increase (15 s period) above the pre-injection control level produced by L-NAME (1 - 20 mgkg^{-1}, i.a) is shown. The increase in discharge produced by L-NAME (10 & 20 mgkg^{-1}) in both normal and arthritic ankle joints was statistically significant when compared with the inactive isomer D-NAME ($p < 0.05$, Mann Whitney U test). Vertical lines represent standard error of the mean.*

The increase in neural discharge evoked by L-NAME was typified by long delay to onset (92 ± 20 s, normal joints; 130 ± 41 s, arthritic joints) and long duration (187 ± 38 s, normal joints; 249 ± 101 s, arthritic joints) of action, there was no statistical significance between normal and srthritic ankle joints. This duration of action was considerably shorter than the duration of the increase in blood pressure observed with L-NAME, greater than 30 minutes, suggesting

that the increase in neural discharge was not related to the vascular efeects of L-NAME (Table 3.1).

	n	control (i.p.s)	peak (i.p.s)	delay (s)	duration (s)	control BP (mm Hg)	peak BP (mm Hg)	duration (min)
NORMAL								
D-NAME 30 $mgkg^{-1}$	4	0.9 ± 0.3	1.0 ± 0.2	-	-			-
SALINE	6	1.4 ± 0.5	2.4 ± 0.4	-	-			-
L-NAME 1 $mgkg^{-1}$	3	1.1 ± 0.1	0.8 ± 0.2	-	-	153	235	30
L-NAME 10 $mgkg^{-1}$	6	1.2 ± 0.3	3.1 ± 0.4	68 ± 23	148 ± 23	110 ± 18	170 ± 20	31
L-NAME 20 $mgkg^{-1}$	6	1.1 ± 0.3	3.6 ± 0.5	92 ± 20	187 ± 38	123 ± 14	180 ±26	38
ARTHRITIC								
D-NAME 30 $mgkg^{-1}$	3	0.5 ± 0.2	0.7 ± 0.1	-	-			-
SALINE	5	1.8 ± 0.5	2.2 ± 0.5	-	-			-
L-NAME 1 $mgkg^{-1}$	3	1.5 ± 0.2	1.8 ± 0.6	-	-	133	233	30
L-NAME 10 $mgkg^{-1}$	4	2.0 ± 0.4	4.2 ± 0.6	59 ± 15	123 ± 14	127 ± 11	187 ± 21	32
L-NAME 20 $mgkg^{-1}$	5	1.1 ± 0.3	4.6 ± 0.7	130 ± 41	249 ± 101	133 ± 13	229 ± 28	45

Table3.1: Summary effects of L-NAME on ankle joint C-fibre mechanonociceptors

All values are mean ± s.e.mean, where possible. In some cases due to technical difficulties, blood pressure recording were not possible.

Neither the NO precursor, L-Arginine (10 - 50 $mgkg^{-1}$, i.a.) nor the inactive isomer D-Arginine (50 $mgkg^{-1}$, i.a.) had any effect on the basal discharge of nociceptive afferents recorded from either normal or arthritic ankle joints; L-Arginine, however, was biologically active, as demonstrated by a fall in blood pressure (Table 3.2).

	n	control (i.p.s)	peak (i.p.s)	control BP (mm Hg)	peak BP (mm Hg)
NORMAL					
D-ARGININE 50 $mgkg^{-1}$	3	0.7 ± 0.24	1.1 ± 0.36	-	-
SALINE	5	1.4 ± 0.48	2.4 ± 0.39	-	-
L-ARGININE 10 $mgkg^{-1}$	3	0.9 ± 0.76	1.3 ± 0.18	108 ± 35	94 ± 32
20 $mgkg^{-1}$	4	0.7 ± 0.09	0.9 ± 0.31	96 ± 4	88 ± 4
50 $mgkg^{-1}$	2	0.4 ± 0.15	0.8 ± 0.1		
ARTHRITIC					
D-ARGININE 50 $mgkg^{-1}$	5	1.3 ± 0.34	1.8 ± 0.42	-	-
SALINE	5	1.8 ± 0.50	2.2 ± 0.50	-	-
L-ARGININE 10 $mgkg^{-1}$	5	1.6 ± 0.46	2.2 ± 0.53	100 ± 8	88 ± 11
20 $mgkg^{-1}$	4	1.9 ± 0.57	1.8 ± 0.55	94 ± 2	80 ± 5
50 $mgkg^{-1}$	2	1.1 ± 0.79	0.9 ± 0.21	92	88

Table 2: Summary effects of L-Arginine on ankle joint C-fibre mechanonociceptors

All values are mean ± s.e.mean, where possible. In some cases due to technical difficulties, blood pressure recording were not possible.

However, co-administration of the NO precursor, L-Arginine (50 $mgkg^{-1}$, i.a.), did attenuate the increase in neural discharge evoked by L-NAME (20 $mgkg^{-1}$, i.a.) in both normal and in arthritic ankle joints (Figure 3.3).

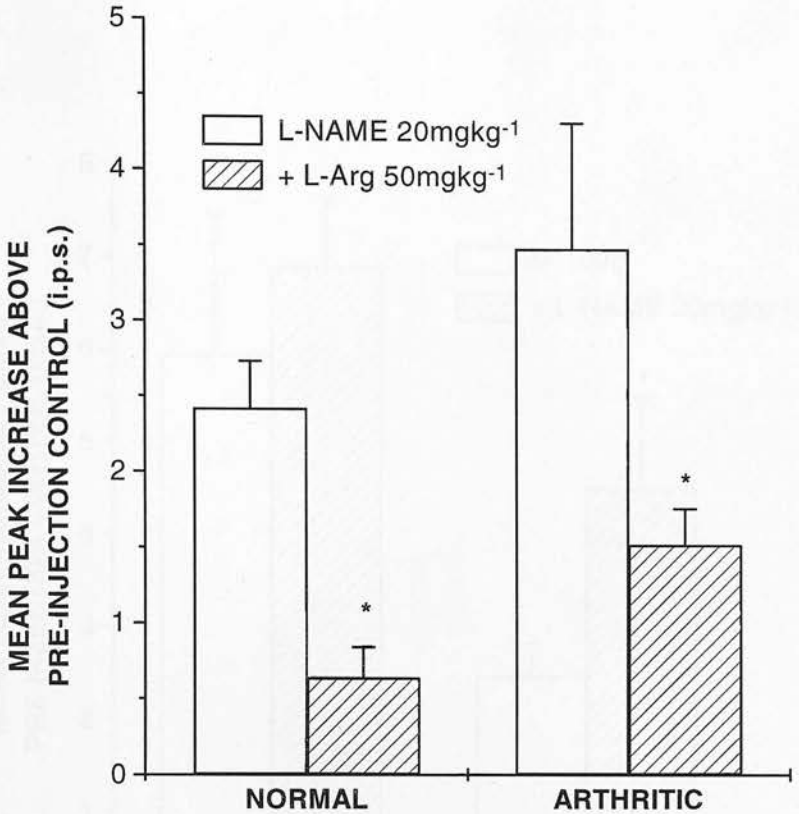


Figure 3.3

L-Arginine reduces L-NAME-induced increase in neural discharge in articular nociceptors recorded from normal and arthritic rat ankle joints.

Each column shows the mean peak increase (15s period) in discharge above the pre-injection control level evoked by L-NAME (20 mgkg^{-1}, i.a) before and after co-administation of L-Arginine (50 mgkg^{-1}, i.a) in normal (n = 6) and arthritic (n = 5) rats. The increase in discharge produced by L-NAME was significantly reduced by L-Arginine in normal and in arthritic rat ankle joints ($p < 0.05$, Wilcoxan matched pairs test). Vertical lines above bars represent the standard error of the mean.*

Effects of L-NAME and L-Arginine on bradykinin-induced activation of articular C-fibre afferents.

Bradykinin (10 μg i.a.) was injected before and 10 minutes after L-NAME (20 mgkg^{-1}, i.a.) when the baseline neural discharge had returned to pre-injection control levels. At this point the blood pressure remained elevated - indicating that NOS was still inhibited. Close intra-arterial injection of L-NAME (20 mgkg^{-1}) increased the responses of nociceptors to

bradykinin (10 μg, i.a.) in arthritic, but not normal ankle joints (Figure 3.4).

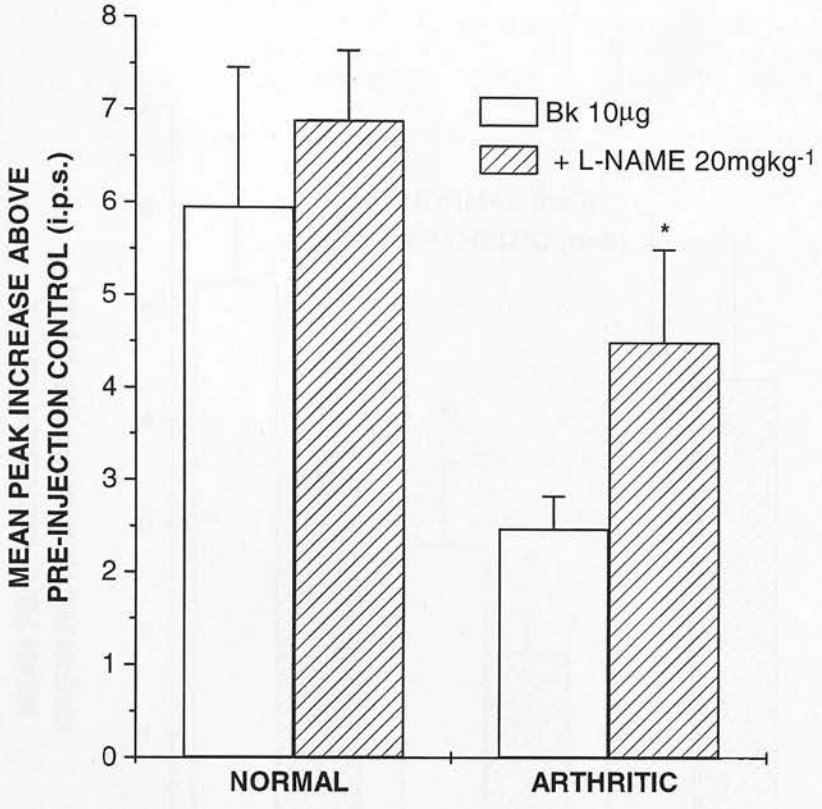


Figure 3.4

L-NAME enhances bradykinin-induced excitation of articular nociceptors in arthritic ankle joints, but not normal joints.

Each bar represents the mean peak increase (15s period) in nociceptor discharge above the pre-injection control level following injection of bradykinin (10μg, i.a.) before and 10 minutes following injection of L-NAME (20 mgkg^{-1}, i.a.) in normal (n=3) and arthritic (n=3) rat ankle joints. The bradykinin-induced excitation was significantly increased following L-NAME in afferents from arthritic ankle joints ($p < 0.05$, Wilcoxan matched pairs test). The basal discharge before both injections of bradykinin was not significantly different (0.9 ± 0.3 & 1.3 ± 0.3 i.p.s in normal joints; 1.7 ± 0.5 & 2.2 ± 0.5 i.p.s in arthritic joints). Vertical lines above the bars represent the standard error of the mean.*

Conversely, bradykinin-induced (30 μg i.a.) activation of articular nociceptive afferents was reduced by co-administration of L-Arginine (50 mgkg^{-1}, i.a.), in both normal and arthritic

ankle joints. The initial response to bradykinin could be recovered within 10 minutes (Figure 3.5). The inactive isomers D-NAME and D-Arginine had no effect on bradykinin-induced activation of nociceptive afferents.

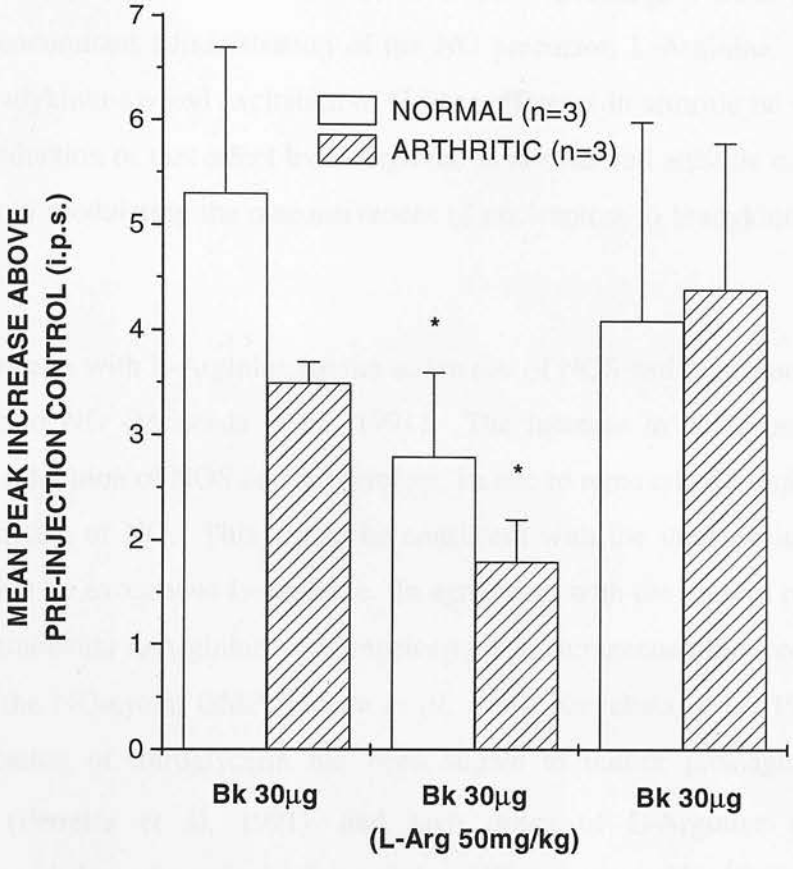


Figure 3.5

L-Arginine reduces bradykinin-induced excitation of articular nociceptors in normal and arthritic ankle joints.

Each bar represents the mean peak increase (15s period) in discharge above a pre-injection control level produced by bradykinin (30µg, i.a.) 10 minutes before, after co-administration of L-Arginine (50 mgkg^{-1}, i.a.) and recovery 10 minutes after L-Arginine in normal and arthritic rat ankle joints. The bradykinin-induced excitation was significantly reduced after L-Arginine ($p < 0.05$, Wicoxan matched pairs test). Vertical lines above bars represent the standard error of the mean.*

3.4 Discussion.

These results provide evidence that endogenous NO may have anti-nociceptive actions in the periphery. The data demonstrates that L-NAME, a NOS inhibitor, but not its inactive isomer, D-NAME, increased nociceptive discharge from joint mechanonociceptors in both normal and arthritic rat ankle joints. The increase in nociceptive discharge evoked by L-NAME was reduced by concomitant administration of the NO precursor, L-Arginine. Furthermore, the increase in bradykinin-evoked excitation of C-fibre afferents in arthritic rat ankle joints by L-NAME and reduction of this effect by L-Arginine in normal and arthritic conditions suggests a role for NO in modulating the responsiveness of nociceptors to bradykinin in both of these states.

L-NAME competes with L-Arginine for the active site of NOS and thus blocks the conversion of L-Arginine to NO (Moncada *et al.*, 1991). The increase in nociceptor discharge seen following the inhibition of NOS could, therefore, be due to removal of a tonic inhibitory (anti-nociceptive) action of NO. This would be consistent with the increase in the reduction in neural discharge by exogenous L-Arginine. In agreement with the present results, it has been shown that intraplantar L-Arginine is antinociceptive in carrageenan-induced hyperalgesia via activation of the NO-cyclic GMP (Duarte *et al,* 1990; Kawabata *at al*, 1994). In addition topical application of nitroglycerin has been shown to reduce prostaglandin E_2-induced hyperalgesia (Ferreira et al, 1991), and high doses of L-Arginine (intraplantar) are antinociceptive in formalin-induced hyperalgesia (Kawabata et al., 1994). L-NAME also increases blood pressure which raises the possibility that the effects on nociceptors are secondary to vascular effects. However, since other vasoconstrictor agents (e.g. endothelin-1 and endothelin-3) had no effect on the activity of nociceptors in our preparation (Kelly *et al*, 1994), this seems improbable.

L-NAME increased the bradykinin-induced excitation of C-fibres innervating arthritic, but not normal, ankle joints. However, L-Arginine attenuated responses to bradykinin in nociceptive afferents from both normal and arthritic ankle joints. This suggests a role for NO in altering the responsiveness of nociceptors to bradykinin. A possible mechanism for this effect could be modulation of the desensitisation to bradykinin responses in nociceptors. There is support

for such a mechanism in the literature : for example, in the neonatal rat tail spinal cord preparation bradykinin-induced activation is attenuated by dibutyryl cGMP and a NO donor, sodium nitroprusside (Dray et al., 1992). Furthermore, NO activates guanylate cyclase, which in turn increases levels of cGMP (McGehee *et al.*, 1992) and activates cGMP. This may result in phosphorylation and desensitision the bradykinin receptor or modulation of the activity of the receptor-coupled ion channel (Dray *et al.*, 1993).

Inflammatory mediators such as bradykinin (Bogle *et al.*, 1991), substance P (Hughes *et al.*, 1990) and cytokines (Pfeilschifter *et al.*, 1992) can release NO. In patients with rheumatoid and osteoarthritis the level of NO (measured in the form of nitrite) in the serum and synovial fluid is elevated (Farrell et al., 1992). It is possible that levels of NO may be insufficient in normal rat ankle joints to reduce the responsiveness of afferents to bradykinin thus explaining the lack of effect of L-NAME under these conditions.

These results suggest of a role for endogenous NO in anti-nociception, acting via tonic inhibition of C-fibre nociceptors and possibly involving generation of cGMP. These findings are also consistent with a role for the L-Arginine-NO pathway in the desensitisation of bradykinin receptors that are on joint mechanonociceptor C-fibres. Increasing the endogenous level of NO may, therefore, reduce the activity of nociceptors and limit pain evoked by noxious stimuli and algesic mediators in the periphery.

4. EFFECTS OF KININS ON NEURAL DISCHARGE FROM C-FIBRES INNERVATING THE RAT KNEE JOINT

4.1 Introduction.

There are several problems associated with studying neural recordings from the ankle joint. The main one of these is the actual anatomy of the joint, the joint is very complex with a lot of small bones. This makes it difficult to inject into the joint itself, and indeed it was only possible to inject inflammatory agents subdermally around the joint resulting in only a superficial inflammation. It was decided that in order to study the effects of individual inflammatory mediators on joint nociceptors it would be much more beneficial if injections could be made directly into the joint space resulting in a more localised inflammation.

The rat knee joint is a much simpler joint to work with, with respect to injections into the joint, and is very similar in anatomy to the human knee joint (Hildebrand *et al.*, 1991). Schaible and his colleagues have investigated in great detail neural recordings from C-fibres innervating the cat knee joint. There are two main articular nerves which innervate the cat knee joint, the medial articular nerve (MAN) and the posterior articular nerve (PAN), both of which contain a high proportion of thin myelinated and unmyelinated fibres (Langford & Schmidt, 1983). In normal joints these fibres have small receptive fields and are only activated by movements outwith the physiological range or by noxious mechanical stimuli. In inflamed joints, however, the receptive field is larger, nerves become sensitised and can be activated by normally non-noxious movements or by relatively low mechanical stimulation (Schaible & Schmidt, 1983a; Schaible & Schmidt, 1983b; Grigg *et al.*, 1986; Schaible & Schmidt, 1985). In addition these nerves can be activated by inflammatory mediators such as bradykinin (Kanaka *et al.*, 1985).

It was decided, therefore, to develop a rat knee joint model for recording from C-fibres. As with the cat knee joint there are two main nerves which contain a large majority of thin myelinated and unmyelinated fibres that innervate the rat knee joint, a MAN which branches from the saphenous nerve and a PAN which branches from the sciatic nerve (Marinozzi *et al.*,

1991) and the MAN has been shown to contain a majority of thin myelinated and unmyelinated fibres with nerve endings within the knee joint (Hildebrand *et al.*, 1991). Records of neural discharge from these articular nerves in the rat has, so far, been limited or not attempted. The aim of these studies was, therefore, to develop a rat model for studying the effects of inflammatory mediators on neural discharge in C-fibres innervating the knee joint. For the purpose of these studies it was decided to record from the MAN as it was easier for experimental design and required less invasive surgery.

4.2 Methods.

As described in section 2.3.

4.3 Results.

The following studies on neural recordings from the rat knee joint were obtained from a total of 80 experiments. Approximately 60% of these experiments were successful in that they satisfied the following criteria: putative C-fibres innervating the rat knee joint were identified initially by manually probing around the knee joint. They were then defined as C-fibres if, the conduction velocity was within the C-fibre range (0.64 ± 0.08 ms^{-1}, range 0.1 - 2 ms^{-1}) and they responded to capsaicin (1 - 3 μg, i.a.) with an increase in neural discharge (Figure 4.1.).

	pre-injection control (i.p.s.)	peak increase(i.p.s.)	delay (s)	duration (s)
capsaicin 1-3μg, i.a. (n=57)	1.4 ± 0.3 (range 0 - 9.7)	34.5 ± 4.5 (range: 3.8 - 102)	1.9 ± 0.4 (range: 0 -7)	11.5 ± 1 (range: 4 -23)

Table 4.1.

Summary of the effects of capsaicin on neural discharge in knee joint C-fibres.

All values are mean ± s.e.mean.

The increase in neural discharge induced by capsaicin had a very short delay to onset and a relatively short duration of action (Table 4.1.). There was no significant difference between the conduction velocities or the response to capsaicin in normal or inflamed knee joints (FCA- or IL-1β-induced). Injection of saline 0.3 ml, i.a. had no significant effect on neural discharge from these fibres.

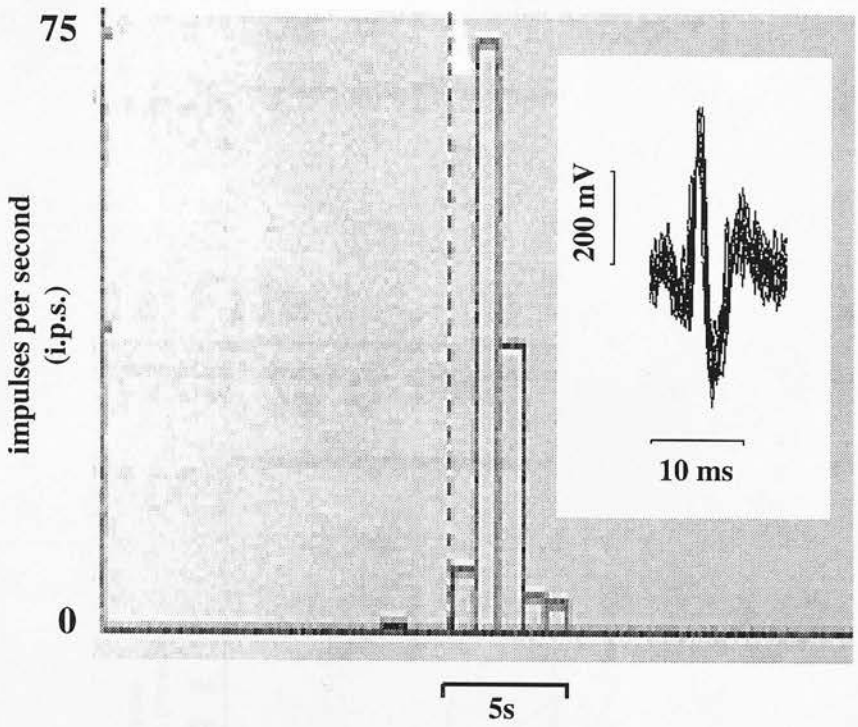


Figure 4.1

Capsaicin increases neural discharge of knee joint C-fibres.

Bargraphs illustrate an example of the increase in discharge evoked by capsaicin (3 μg, i.a. at broken line) in an untreated knee joint (n = 1), each bar represents 1s. The inset illustrates 20 fast oscilloscope sweeps of the activated unit.

Recordings were made from arthritic knee joints at least 14 days after injection of FCA when the inflammation, as measured by callipers, was 17 ± 1.7 % greater in diameter when compared to the contralateral (uninjected) knee joint.

Effects of bradykinin on the neural discharge of C-fibres in normal and arthritic knee joints.

Bk (3 - 30μg, i.a.) produced a dose dependent increase in neural discharge from C-fibres in both normal and arthritic knee joints. Although the peak increase observed in normal knee joints had a tendency to be greater than that seen in arthritic knee joints (30 μg, i.a. produced a peak increase of 8.5 ± 2.2 i.p.s. above pre-injection control discharge 2.4 ± 1.2 i.p.s in arthritic knee joints compared to 23 ± 10.7 i.p.s. above pre-injection control discharge 3.6 ± 1.7 i.p.s. in normal knee joints), this did not reach statistical significance and there was no difference in

the total number of impulses above the pre-injection control in both normal and arthritic knee joints (Figure 4.2.).

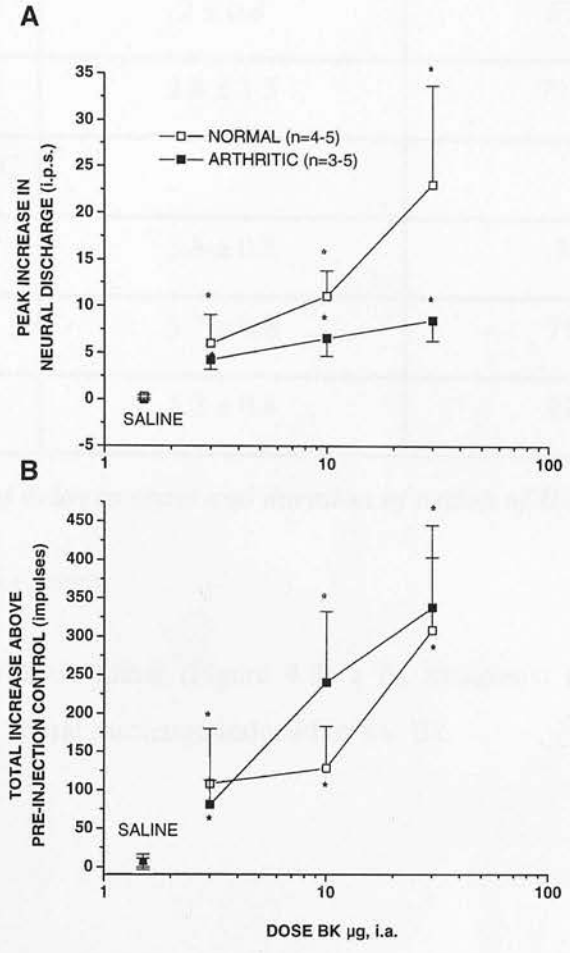


Figure 4.2

Bradykinin increases in neural discharge in normal and arthritic knee joints.

*(A) The peak increase in neural discharge induced by Bk, i.a. in normal knee joints and arthritic knee joints. (B) The total increase in neural discharge induced by Bk, i.a. in normal and arthritic knee joints. All values are mean ± s.e.m., * $p < 0.05$ compared to saline, Mann Whitney U test.*

The increase in neural discharge induced by Bk was typified by a short duration to onset and a relatively long duration of action (Table 4.2.)

NORMAL	delay to onset (s)	duration of action (s)
3 μg	9 ± 6.4	84 ± 57
10 μg	2 ± 0.8	67 ± 38
30 μg	2.8 ± 1.5	79 ± 116
ARTHRITIC		
3 μg	3.8 ± 0.8	36 ± 9
10 μg	3.7 ± 0.8	71 ± 14
30 μg	3.2 ± 0.8	91 ± 20

Table 4.2. Summary of delay to onset and duration of action of Bk

All values are mean ± s.e.mean.

In both normal and arthritic joints (Figure 4.3) a B_2 antagonist icatibant (10 μgkg^{-1}, i.a.) blocked the increase in neural discharge induced by i.a. Bk.

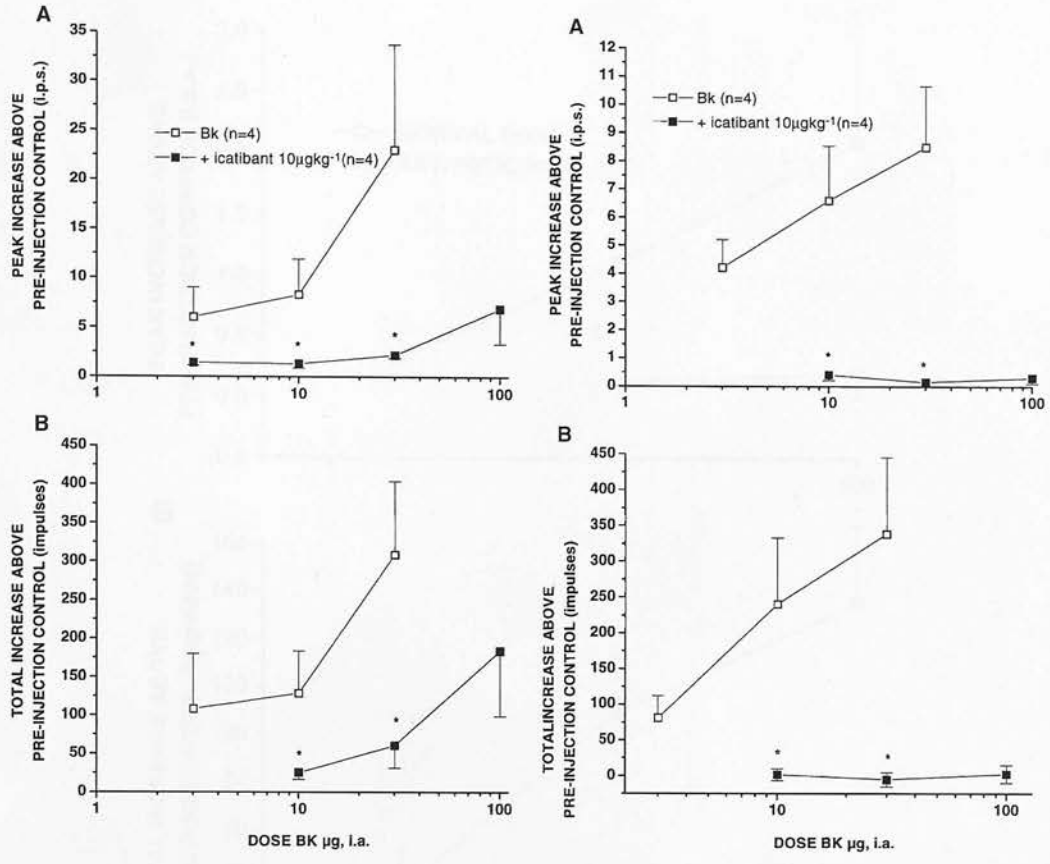


Figure 4.3

Bradykinin-induced increase in neural discharge in normal and arthritic knee joints is blocked by icatibant.

*(A) Peak increase in neural discharge induced by i.a. Bk alone and after icatibant, 10 μgkg^{-1}. (B) Total increase in neural discharge induced by i.a. Bk alone and after icatibant, 10 μgkg^{-1}, i.a.. Graphs on the left show normal joints and on the left arthritic joints. All values are mean ± s.e.m., * $p < 0.05$ compared to Bk alone, Mann Whitney U test.*

The kinin B_1 receptor agonist, desArg^9-Bk increased neural discharge from C-fibres innervating arthritic knee, but not normal knee joints. DesArg^9-Bk, 30μg and 100μg, i.a., produced a peak increase in neural discharge (1.1 ± 0.07 i.p.s. above pre-injection control discharge 0.8 ± 0.2 i.p.s. and 2.1 ± 0.8 i.p.s. above pre-injection control discharge 1.2 ± 0.3 i.p.s., respectively) which, although not as pronounced as that produced by Bk, was significantly greater than that produced by saline (Figure 4.4.).

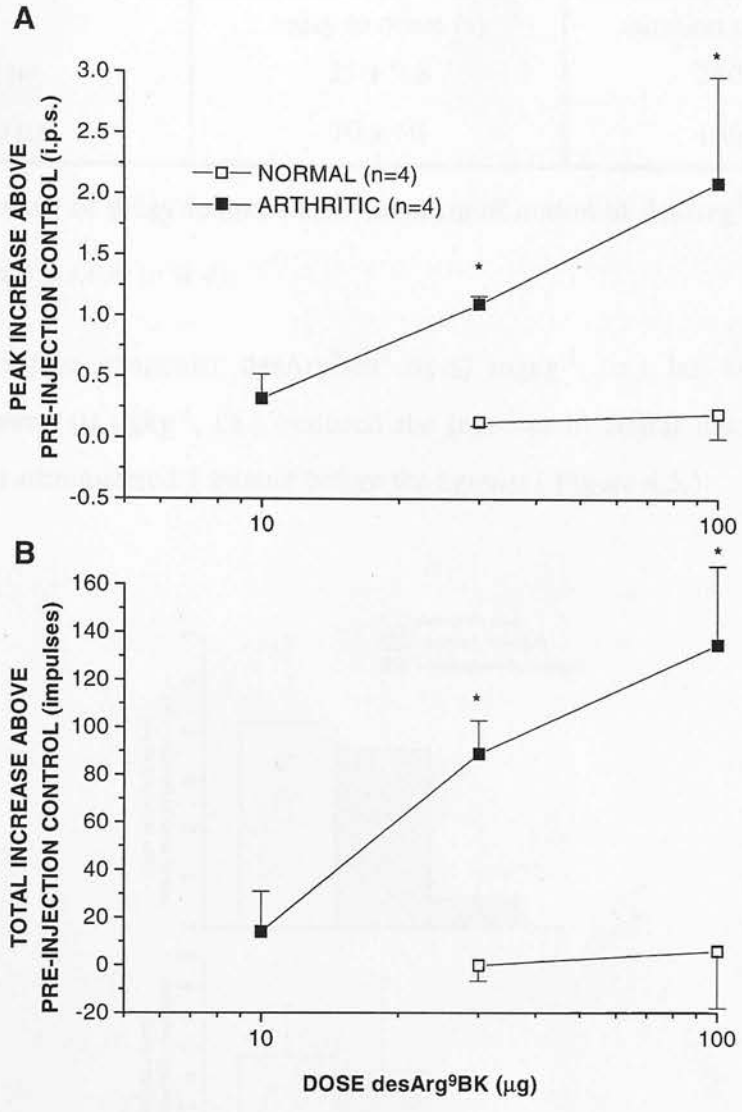


Figure 4.4

DesArg^9-Bk increases neural discharge in arthritic knee joints , but not normal knee joints.

*(A) The peak increase in neural discharge induced by desArg^9-Bk, i.a. in normal and arthritic knee joints. (B) The total increase in neural discharge induced by desArg^9-Bk, i.a. in normal and arthritic knee joints. All values are mean ± s.e.m., * $p < 0.05$ compared to normal, Mann Whitney U test.*

The increase produced was typified by a longer delay to onset to action and a longer duration than that evoked by Bk (Table 4.3.).

	delay to onset (s)	duration of action (s)
30 µg	25 ± 9.8	230 ± 39
100 µg	70 ± 50	166 ± 44

Table 4.3. Summary of delay to onset and duration of action of desArg^9-Bk.

All values are mean ± s.e.m (n = 4).

The kinin B_1 receptor antagonist, desArg^{9}leu^8-Bk (1 mgkg^{-1}, i.a.), but not the B_2 receptor antagonist, icatibant (10 µgkg^{-1}, i.a.), reduced the increase in neural discharge induced by desArg^9-Bk when administered 1 minute before the agonist (Figure 4.5.).

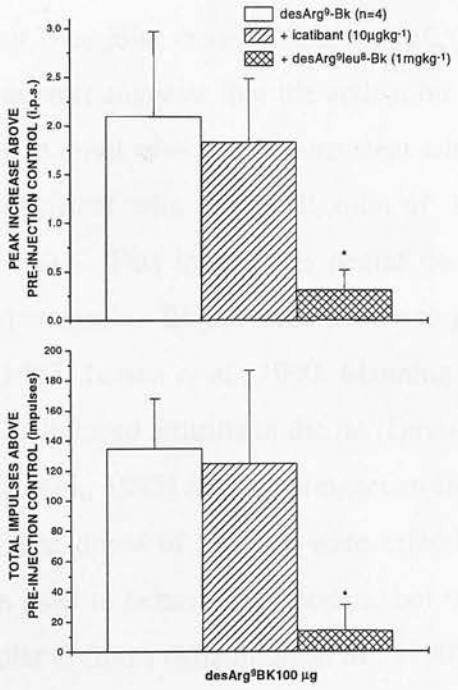


Figure 4.5

des Arg^9 Bk-induced increase in neural discharge in normal knee joints is blocked by B_1 but not B_2 antagonists..

*(top panel) The peak increase in neural discharge induced by desArg^9-Bk, i.a., with icatibant 10µgkg^{-1}, i.a. and desArg^{9}leu^8-Bk 1mgkg^{-1}, i.a. (lower panel) The total increase in neural discharge induced by desArg^9-Bk, i.a. with antagonists as above. All values are mean ± s.e.m., * $p < 0.05$ compared to open bars, Wilcoxan matched pairs test.*

4.4 Discussion.

These results demonstrate a novel model for recording neural discharge from C-fibres innervating the medial side of the rat knee joint. This model proved useful for assessing the actions of kinins in both normal and inflamed knee joints. In both normal and arthritic knee joints Bk produced an increase in neural discharge which was mediated via kinin B_2 receptors. On the other hand desArg^9-Bk only produced an increase in neural discharge in inflamed knee joints, an action which was mediated via kinin B_1 receptors. The antagonism of the responses to Bk and desArg^9-Bk by B_2 and B_1 kinin receptor antagonists, respectively, demonstrate that the responses to these agonists were mediated by their respective receptors.

The increase in neural discharge evoked by bradykinin from C-fibres was comparable to that observed previously in the cat knee joint model (Kanaka *et al.*, 1985). The antagonism of Bk responses by a kinin B_2 antagonist suggests that the activation of C-fibres by Bk was via B_2 receptors and the short delay to onset of action is consistent with a direct action of Bk on the C-fibre terminals. This is consistent with the localisation of kinin B_2 receptors on sensory neurons (Steranka *et al.*, 1988). This increase in neural discharge evoked by Bk can be correlated to behavioural hyperalgesia. Bk has been shown to produce pain and hyperalgesia in humans (Whalley *et al.*, 1987; Jansen *et al.*, 1990; Manning *et al.*, 1991) and a number of animal models including FCA-induced arthritis in the rat (Davis *et al.*, 1994), paw withdrawal thresholds in the rat (Khasar *et al.*, 1993) and in carrageenan-induced inflammation in the rat paw (Carey *et al.*, 1990). The doses of Bk that were effective in the present studies are somewhat higher than those used in behavioural models, but were within the same range as those used to activate testicular C-fibres (Mizumura *et al.*, 1990) and in cat knee joint C-fibres (Kanaka *et al.*, 1985).

The observation that kinin B_1 agonists only increased neural discharge in inflamed knee joints is consistent with the evidence that B_1 kinin receptors are rarely, if at all, present under normal physiological conditions. In fact, originally B_1 mediated effects were only seen in vascular smooth muscle preparations *in vitro* and in these preparations responses to desArg^9-Bk were minimal initially, but increased over time (Regoli *et al.*, 1977). Subsequent studies demonstrated that this increase in responsiveness to desArg^9-Bk could be mimicked by pro-

inflammatory agents including lipopolysaccharide and cytokines (Deblois *et al.*, 1991). More recently, kinin B_1 receptors have been found to be involved in inflammatory hyperalgesia (Perkins *et al.*, 1992).

Whether the B_1 mediated increase in neural discharge is via a direct action on the sensory neuron cannot be determined from the present experiments, although the longer delay to onset might suggest that the B_1 receptors are not localised on the nociceptors. Indeed, Davis et al., 1996 found no evidence of B_1 receptors on dorsal root ganglion cells in rats which have been pre-treated with FCA. It is, therefore, more likely that B_1 receptors are induced on non-neuronal cells or inflammatory cells, activation of these leading to the release mediators which then act directly on the nociceptor.

Kinin B_1 and B_2 receptor antagonists have been shown to be antinociceptive in a number of inflammatory hyperalgesia models including FCA-induced arthritis in the rat knee (Davis & Perkins, 1994A), UV-induced thermal hyperalgesia (Perkins *et al.*, 1993) and the formalin model in the rat (Correa & Calixto, 1993). However in the present model, there was no effect of either desArg^{9}leu^8-Bk or icatibant on the spontaneous neural discharge. This agrees with studies done in the arthritic cat knee joint where neither kinin B_1 nor B_2 antagonists had any effect on the on-going neural discharge (Messlinger *et al.*, 1993). However, in the present model this may be difficult to evaluate as the relatively low background discharge 1 - 1.5 i.p.s. of individual C-fibres makes it difficult to measure decreases in neural discharge. In order to observed such a decrease in neural discharge with kinin antagonists it may be necessary to induce C-fibre activity , possibly by studying a mechanical- or movement-induced increase in neural discharge.

The present results demonstrate that this novel model is useful in studying the effects of kinins on C-fibres innervating the rat knee joint and relating these effects to behavioural hyperalgesia models. In addition these findings provide further evidence that Bk acts via B_2 receptors on C-fibres to produce hyperalgesia and that in inflammatory conditions kinin B_1 receptors can be induced which, upon activation by selective B_1 agonists results in C-fibre activation.

5. THE ROLE OF IL-1β IN HYPERALGESIA AND MODULATION OF EFFECTS OF KININS IN HYPERALGESIA : ELECTOPHYSIOLOGICAL AND BEHAVIOURAL STUDIES.

5.1 Introduction.

As previously discussed, cytokines have been shown to induce kinin B_1 receptor-mediated actions in vascular smooth muscle and B_1 kinin receptor-mediated hyperalgesia *in vivo* (Deblois *et al.*, 1989; Perkins & Kelly, 1994; Davis & Perkins, 1994). A role for cytokines in inducing B_1 kinin receptors is consistent with the increased levels of several cytokines that has been observed in conditions such as rheumatoid arthritis in man. Cytokines have a multitude of pro-inflammatory actions, and very low levels are required to produce a biological action. Of particular interest for these studies is IL-1β. IL-1β induces B_1 receptors in vascular smooth muscle an action which appears to require *de novo* synthesis as it is blocked by an inhibitor of protein synthesis, cyclohexamide (Deblois *et al.*, 1991).

Local injection of IL-1β has been shown to reduce withdrawal thresholds in paw pressure test (Ferreira *et al.*, 1988) and produce both a thermal and mechanical hyperalgesia, an effect involving both kinin B_1 and B_2 receptors (Perkins & Kelly, 1994; Davis & Perkins, 1994). IL-1β can also release a variety of inflammatory mediators from inflammatory cells including prostanoids and kinins and has been shown to upregulate kinin B_1 and B_2 receptors on smooth muscle cells and fibroblasts and can enhance responses to kinins (Galizzi *et al.*, 1994; Lerner & Modeer, 1991).

The aim of these studies was to investigate the effects of local injection of IL-1β on spontaneous and Bk-evoked neural discharge and also to investigate the role of IL-1β in inducing the B_1-mediated increase in neural discharge which was observed in arthritic knee joints. Behavioural hyperalgesia experiments were also carried out in order to try and correlate the *in vitro* findings to the situation *in vivo*.

5.2 Methods.

Neuronal experiments were carried out as described in section 2.3. Dose responses of Bk and desArg^9-Bk were obtained and then the nerve was then lowered to allow i.art. injection of either IL-1β, boiled IL-1 or IL-1ra. The nerve was then raised and neural discharge was subsequently measured for 1 hour after i.art. injection when further dose responses to kinins were obtained. The same fibres were observed before and after i.art injection by carefully observing the size and shape of the activated spikes by use of an oscilloscope.

Behavioural experiments were as described in section 2.4. Dose response curves with local injections of IL-1β, Bk and desArg^9-Bk were obtained. To establish the effects of IL-1β on kinin activity, concomitant administration of kinins and cytokines together with antagonists, when used, were administered locally.

5.3 Results.

In the behavioural experiments discussed in this section, the pre-injection control values were as follows. The load tolerated before i.art. injection was 94.6 ± 0.7g (range: 71 - 146g, n = 355). The withdrawal latency in the thermal hyperalgesia model before i.pl. injection was 10 ± 0.2 s (range: 6.7 - 17.1s, n = 136).

Effects of IL-1ß on the neural discharge of C-fibres and behavioral hyperalgesia.

IL-1ß (10 - 100 units, i.art.) increased the spontaneous on-going neural discharge in C-fibres innervating the rat knee joint (Figure 5.1). The increase in neural discharge had two phases, an initial peak 8 to10 minutes after injection when the discharge increased from 0.3 ± 0.1 i.p.s. to 1.5 ± 0.2 i.p.s., and a delayed peak 40 minutes after injection where discharge increased from 0.48 ± 0.1 i.p.s. to 1.7 ± 0.5 i.p.s. (100 u i.art.).

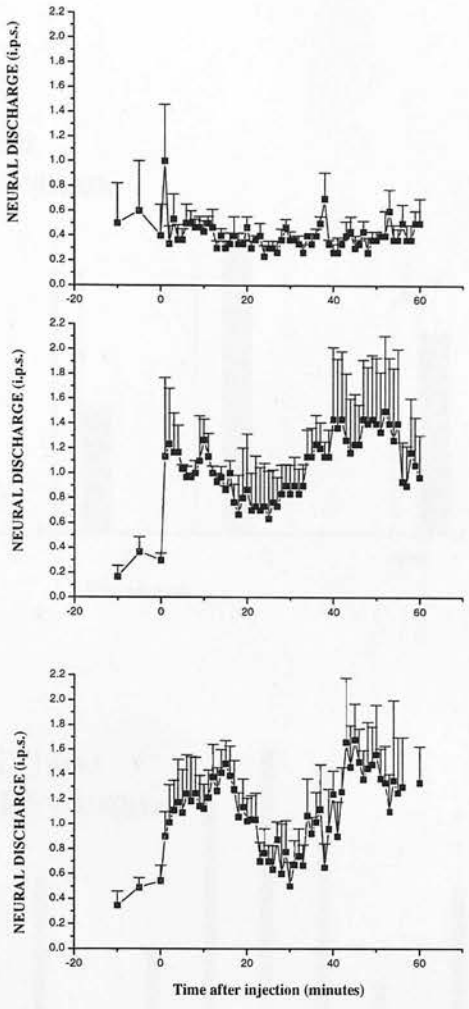


Figure 5.1

IL-1ß increases neural discharge in knee joint C-fibres.

IL-1ß 1 u (top graph, n = 3), 10 u (middle graph, n = 3) and 100 u (bottom graph, n = 9). All values are expressed as mean ± s.e.mean.

Due to the temporal variation between individual experiments, statistical analysis was done on the total increase in neural discharge following intra-articular injection. For this analysis the assumption was made that the pre-injection control discharge would remain constant throughout. This is valid as (inactivated) boiled IL-1β did not induce a significant increase in neural discharge (Figure 5.2), whereas, IL-1β, 100u, i.art., produced a significant increase in neural discharge when compared to the pre-injection control (Figure 5.2)

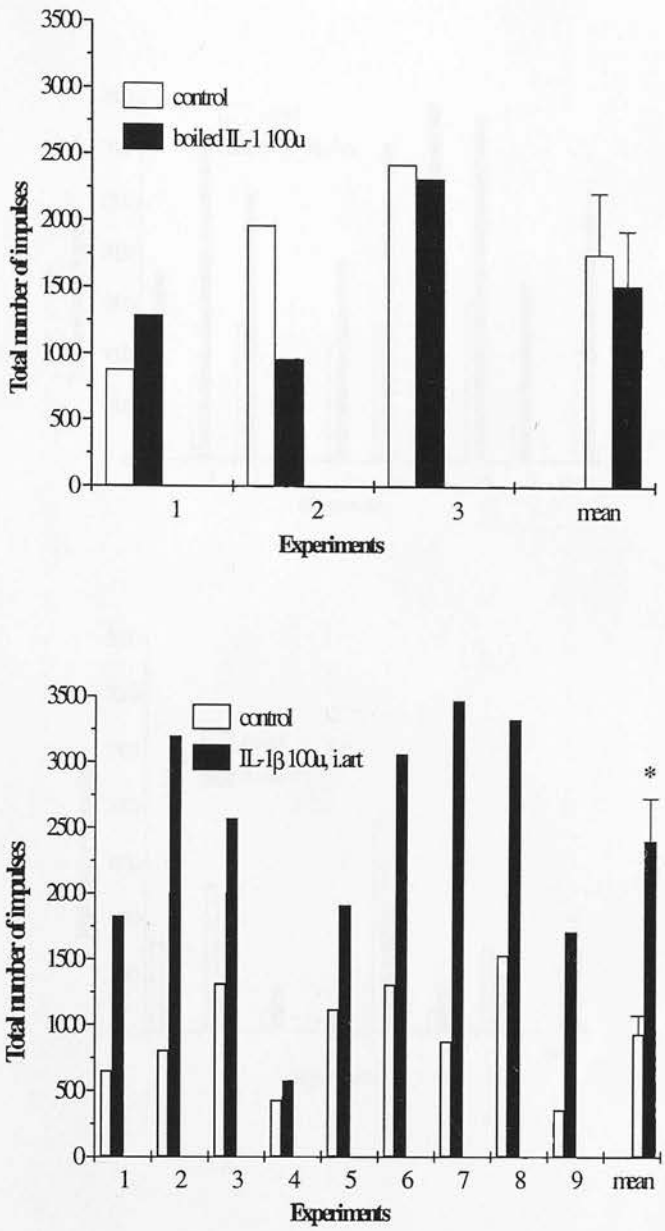


Figure 5.2

IL-1β increases neural discharge in knee joint C-fibres.

*(top graph) Total impulses following boiled IL-1β compared to control and (lower graph) IL-1β 100 u. Bar graphs show individual experiments with the corresponding mean ± s.e.mean, * $p < 0.05$ compared control, Wilcoxan matched pairs test.*

The increase in neural discharge produced by IL-1ß (100u, i.art.) was blocked by co-injection

of IL-1ra (0.1μg, i.art.) [Figure 5.3.].

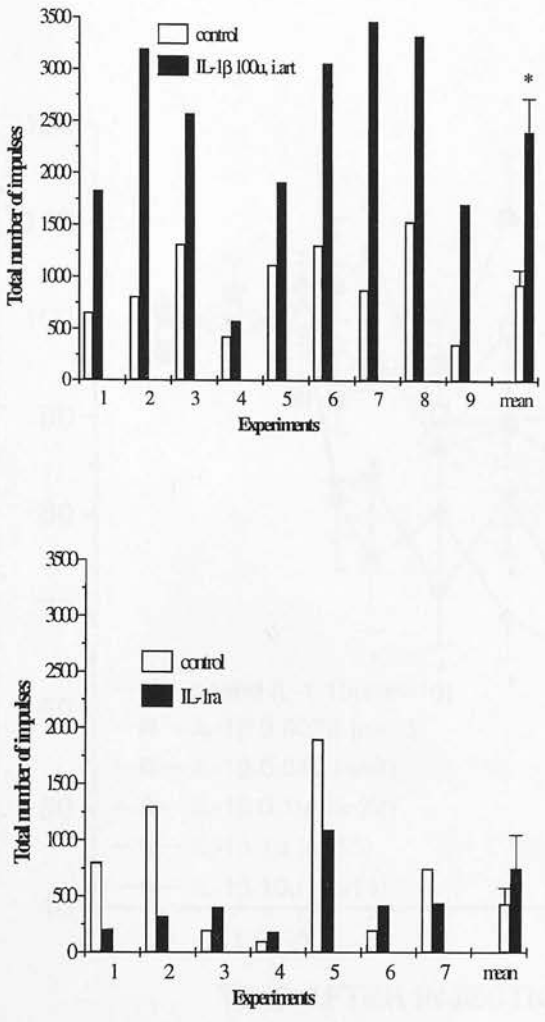


Figure 5.3

IL-1β-induced increase in neural discharge is blocked by IL-1ra.

*(top graph) Total impulses following IL-1β compared to control and (lower graph) IL-1β co-injected with IL-1ra 0.1 μg. Bar graphs show individual experiments with the corresponding mean ± s.e.mean, * $p < 0.05$ compared control, Wilcoxan matched pairs test.*

IL-1β (0.1 - 10 u, i.art.), but not boiled IL-1β (10 u) reduced the load tolerated by the injected knee (Figure 5.4.). The decrease in load tolerated had reached significance 1h after injection when the load tolerated had fallen to 83.7 ± 9.3 % (1u, i.art.) and 75.5 ± 7.3 % (10 u, i.art) of

the pre-injection control. The maximum hyperalgesia was 5h after injection when the load tolerated had fallen to 80.2 ± 3.8 % (0.1u), 57.9 ± 4.9 % (1 u) and 62.4 ± 5.6 % (10 u).

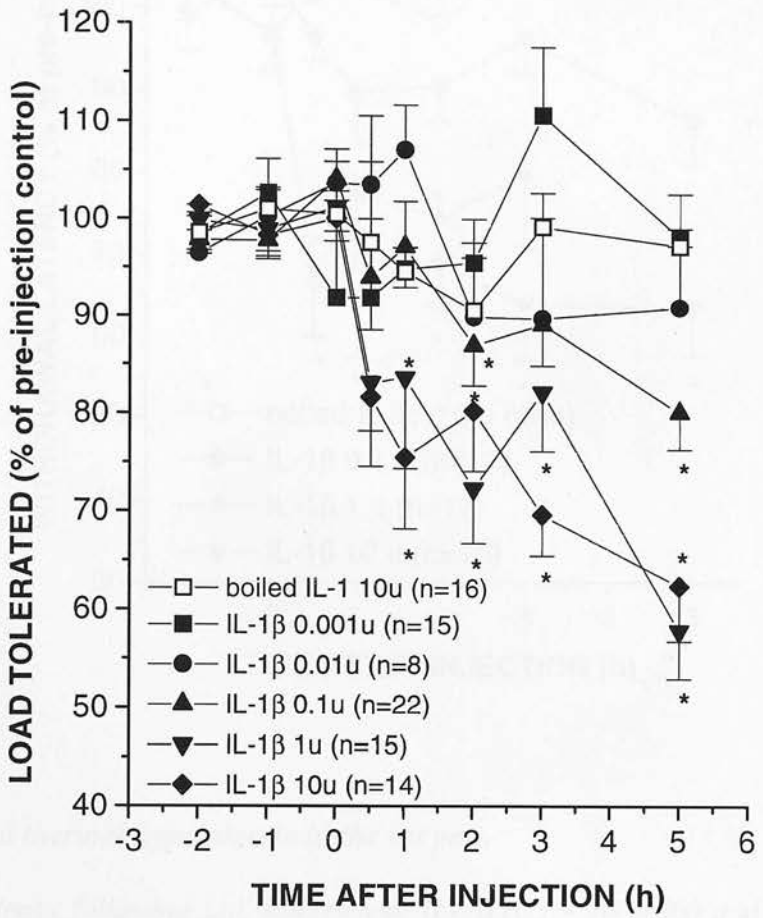


Figure 5.4

IL-1β induces a mechanical hyperalgesia in the rat knee joint.

*Load tolerated following i.art. injection of IL-1β (0.001 - 10 units) and inactivated IL-1β. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

IL-1β (1 - 10 u,i.art.) but not boiled IL-1β also reduced the latency of paw withdrawal to a focused radiant heat lamp. This was significant 30 minutes after intra-plantar injection when the withdrawal latency had fallen to 66.8 ± 7.1 % (1 u) and 71.6 ± 13.5 % (10 u) of the pre-injection control. A maximum reduction in withdrawal latency of 54.2 ± 7.9 % was observed 1 h after injection (Figure 5.5.).

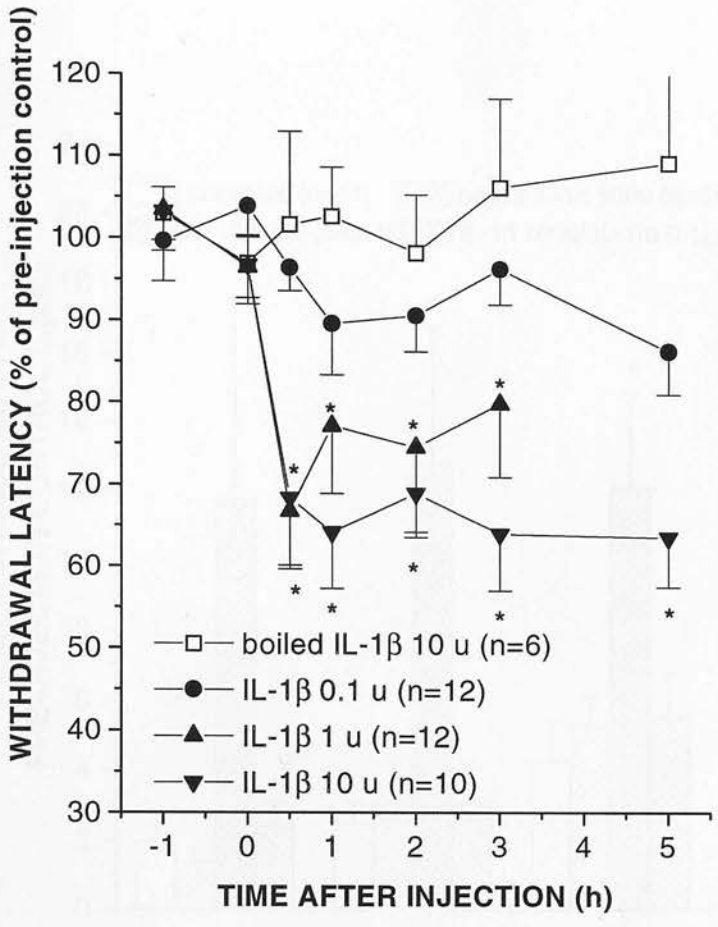


Figure 5.5

IL-1ß induces a thermal hyperalgesia in the rat paw.

*Withdrawal latency following i.pl. injection of IL-1β (0.1 - 10 units) and boiled IL-1β. All values are expressed as means ± s.e.mean, * $p < 0.05$ compared to pre-injection control.*

Effects of IL-1β on kinin-mediated actions on C-fibres and in behavioural hyperalgesia.

Kinin B_2-mediated effects.

Bk-induced increase in neural discharge was enhanced 1h following i.art. injection of IL-1β 100 u but not boiled IL-1β. This was evident at all 3 doses (3, 10 and 30µg, i.a.). Co-injection of IL-1ra 0.1 µg blocked the increased responsiveness to Bk induced by IL-1β 100 u (Figure 5.6.).

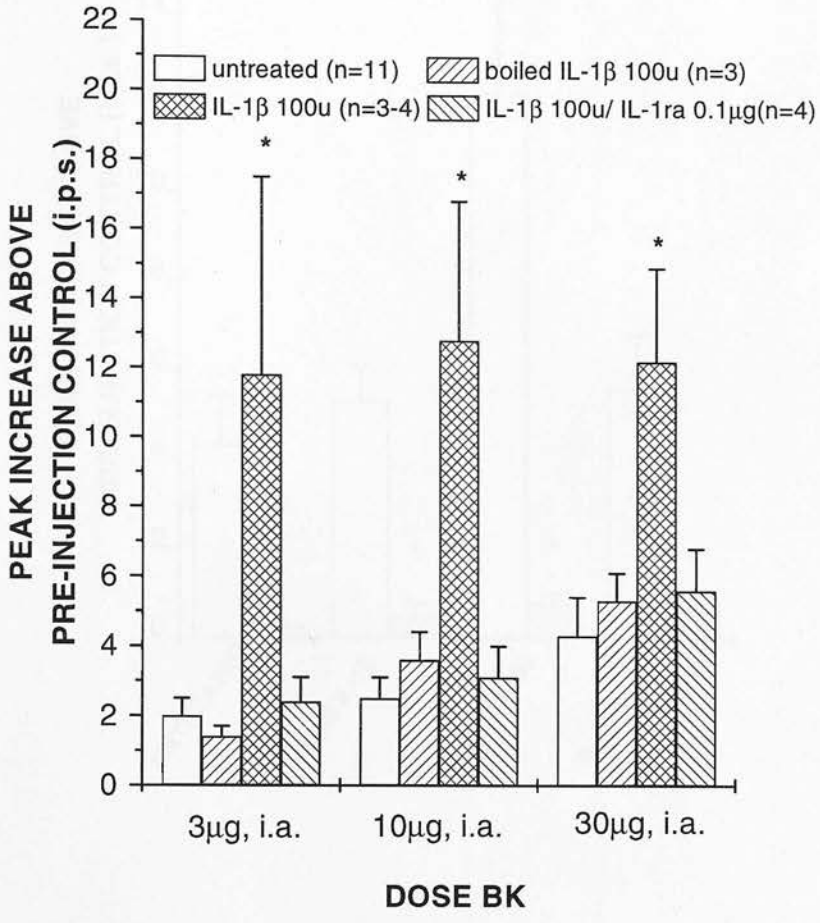


Figure 5.6

IL-1β enhances Bk-induced increase in neural discharge in knee joint C-fibres, which is blocked by IL-1ra.

*Effects of Bk (3 - 30 μg, i.a.) is shown in naïve joints, or after intra-articular injection of boiled IL-1β, IL-1β 100 u and IL-1β 100 u with IL-1ra 0.1 μg. All values are mean ± s.e.mean, * $p < 0.05$ compared to boiled IL-1β-treated joints, Mann Whitney U test.*

In all joints (naïve, boiled IL-1β-treated, IL-1β-treated, or IL-1β with IL-1ra) the Bk-induced increase in neural discharge from articular C-fibres was blocked by injection of a kinin B_2 antagonist, icatibant (10 $\mu g kg^{-1}$, i.a.)[Figure 5.7.].

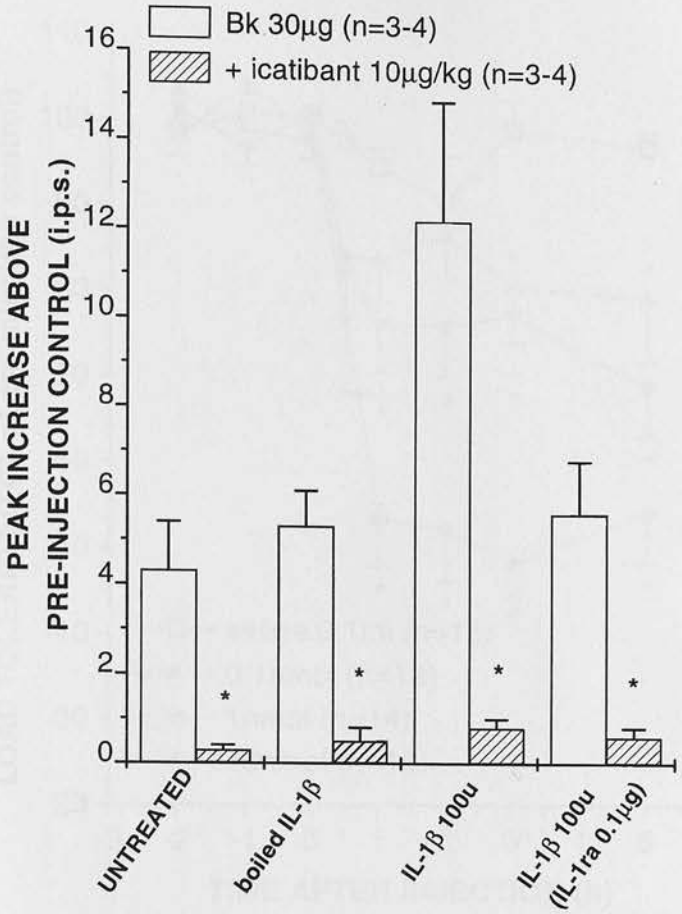


Figure 5.7

Bk-induced increase in neural discharge in C-fibres innervating untreated or injected knee joints is blocked by icatibant.

*Effects are shown for bradykinin 30 µg, i.a. before and following icatibant 10 $\mu g kg^{-1}$, i.a.. All values are mean ± s.e.mean, * $p < 0.05$ compared to bradykinin effects before injection of the antagonists, Mann Whitney U test.*

Intra-articular injection of Bk (0.1 - 10 moles) induced a dose dependent reduction in load tolerated by the injected knee. This was significant 1 h after injection when the load tolerated had fallen to (83.8 ± 6.9 %) 0.1 nmol, (76.2 ± 5.6 %) 1 nmol and (53.5 ± 5.6 %) 10 moles and was maintained for up to 5 h post-injection : (79.6 ± 7.5 %) 0.1 nmol, (69.2 ± 5.8 %) 1 nmol and (54.4 ± 6.0 %) 10 nmol (Figure 5.8.).

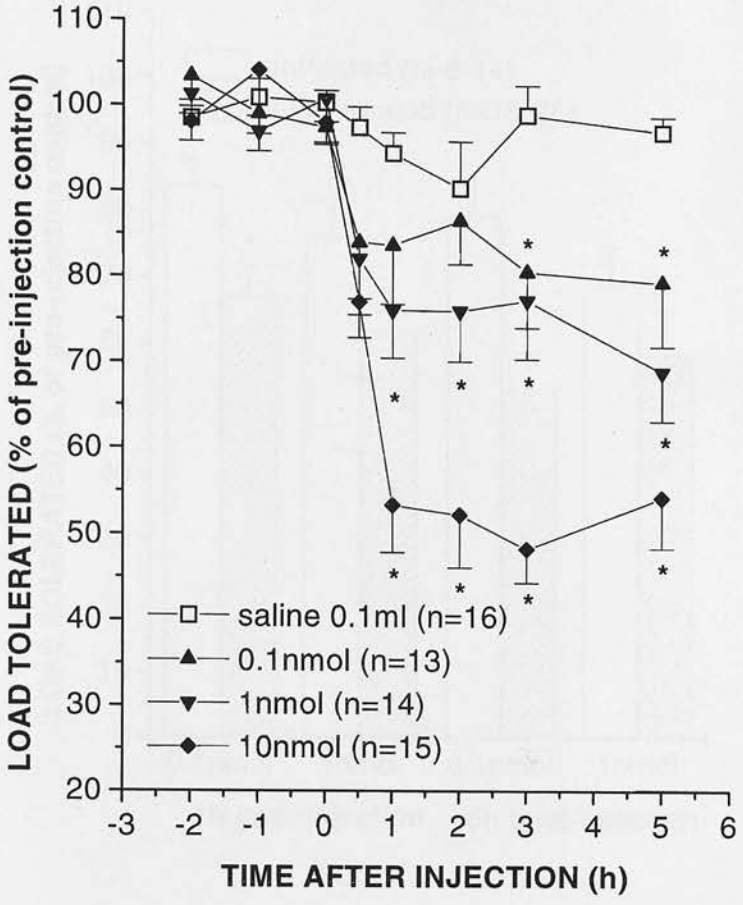


Figure 5.8

Bk induces a mechanical hyperalgesia in the rat knee joint.

*Effects of i.art. Bk (0.1 - 10 nmol) on load tolerated by the injected knee are shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

The reduction in load tolerated induced by Bk (0.1 and 1 nmole, i.art.) was enhanced following co-injection of a sub-threshold dose of IL-1β (0.001 u) with a further reduction of 12.4 % (0.1nmole) and 26.5 % (1nmole). However, 5 h after Bk an enhanced reduction of 24.2 % was only observed at the lower dose (Figure 5.9.).

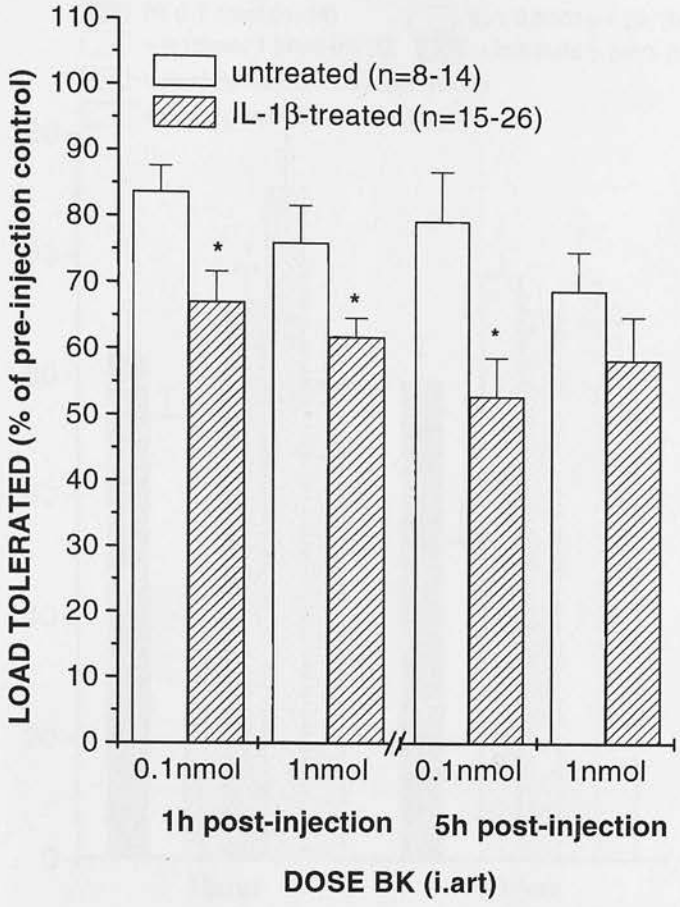


Figure 5.9

IL-1ß enhances the mechanical hyperalgesia induced by Bk.

*The load tolerated following i.art. injection of Bk alone or co-injection with IL-1ß 0.001 u is shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

The reduction in load tolerated induced by co-injection of Bk (0.1 nmole, i.art.) with sub-threshold dose of IL-1β (0.001 u) was blocked by co-injection of kinin B_1 and B_2 receptor antagonists. Icatibant (1 and 5 pmoles, i.art.), a kinin B_2 antagonist, blocked the reduction in load tolerated by the injected knee 5 h following injection, whereas only the higher dose was effective 1 h following injection. DesArg^{9}leu^8-Bk (0.5 and 1 nmole, i.art.) also blocked the reduction in load tolerated by the injected knee 5 h following injection, whereas only the lower dose had any effect 1 h following injection (Figure 5.10.).

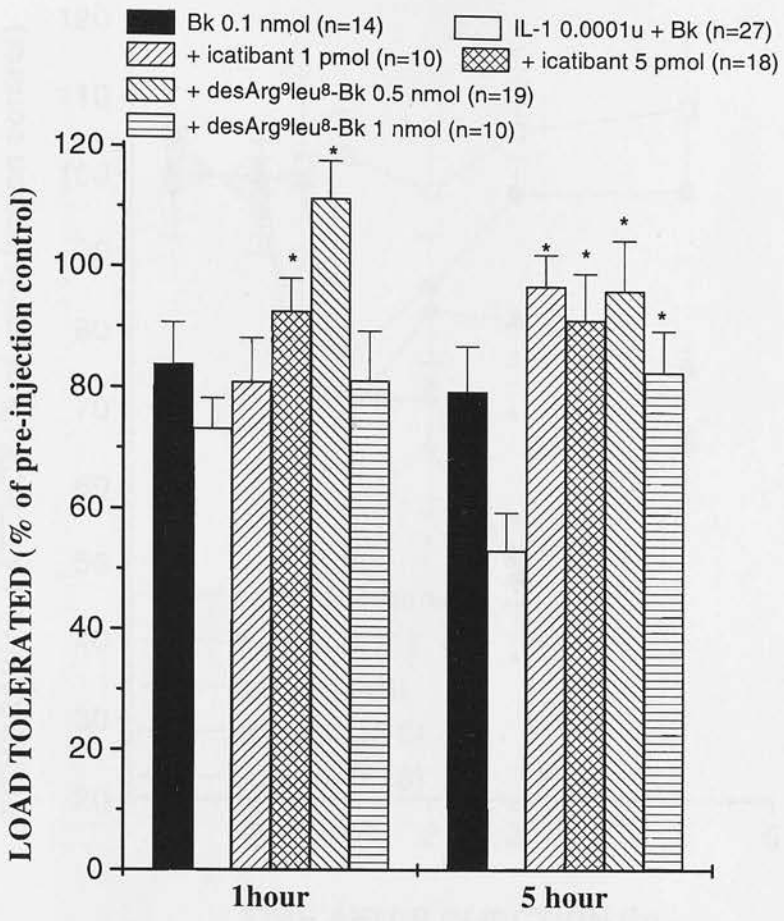


Figure 5.10

Kinin B_1 and B_2 receptor antagonists block the enhanced mechanical hyperalgesia by Bk induced by IL-1ß in the rat knee joint.

*Bk (0.1 nmole) with sub-threshold IL-1β (0.001 u) alone (open bars, n =27), and with icatibant (1pmole)(right hatched bars, n =10), Icatibant (5 pmoles, n =18)(cross hatched bars, n = 19), desArg^9 leu^8-Bk (0.5nmoles)(left hatched bars, n = 19), and desArg^9 leu^8-Bk (1 nmole)(horizontal bars, n = 10). All values are mean ± s.e.mean. *p < 0.05, compared to open bars.*

Bk (1 - 50 nmoles, i.pl) also produced a dose-dependent reduction in withdrawal latency to focused radiant heat. This reduction was significant 30 min after injection and was maintained for the duration of the time course (5 h) (Figure 5.11.).

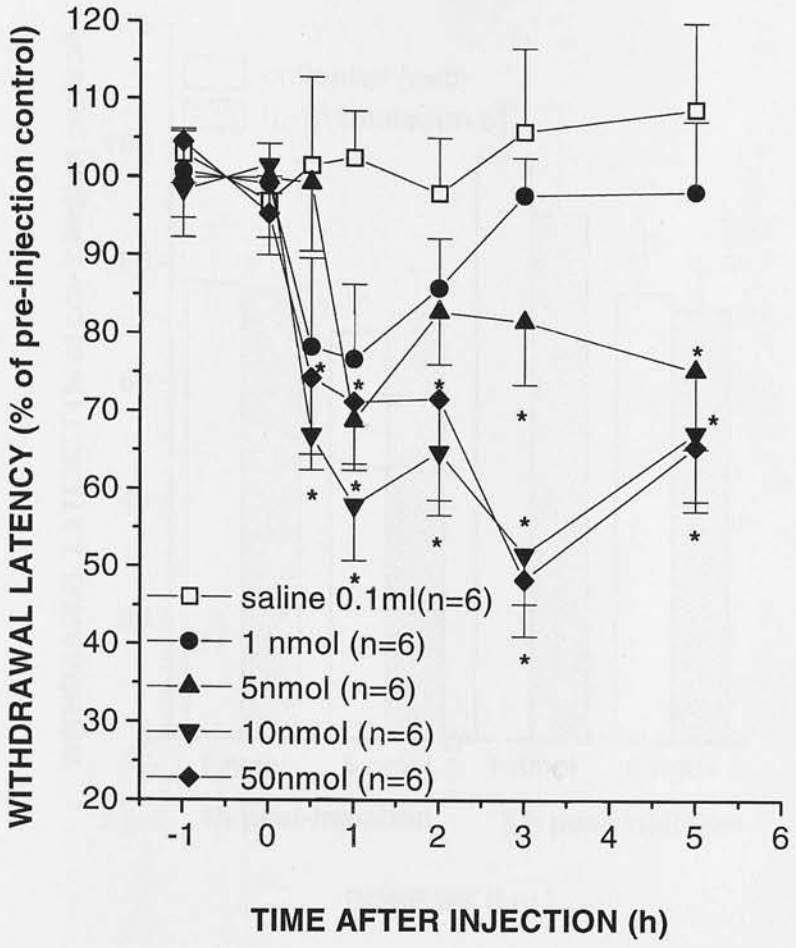


Figure 5.11

Bk induces a thermal hyperalgesia in the rat paw.

*Withdrawal latency following i.pl. injection of Bk (1 - 50 nmol) is shown. All values are expressed as means ± s.e.mean, * $p < 0.05$ compared to pre-injection control.*

Co-injection of a sub-threshold dose of IL-1β (0.1 u, i.pl.) did not enhance the reduction in withdrawal latency, induced by Bk (1 & 5 nmoles), to a thermal stimulus (Figure 5.12.).

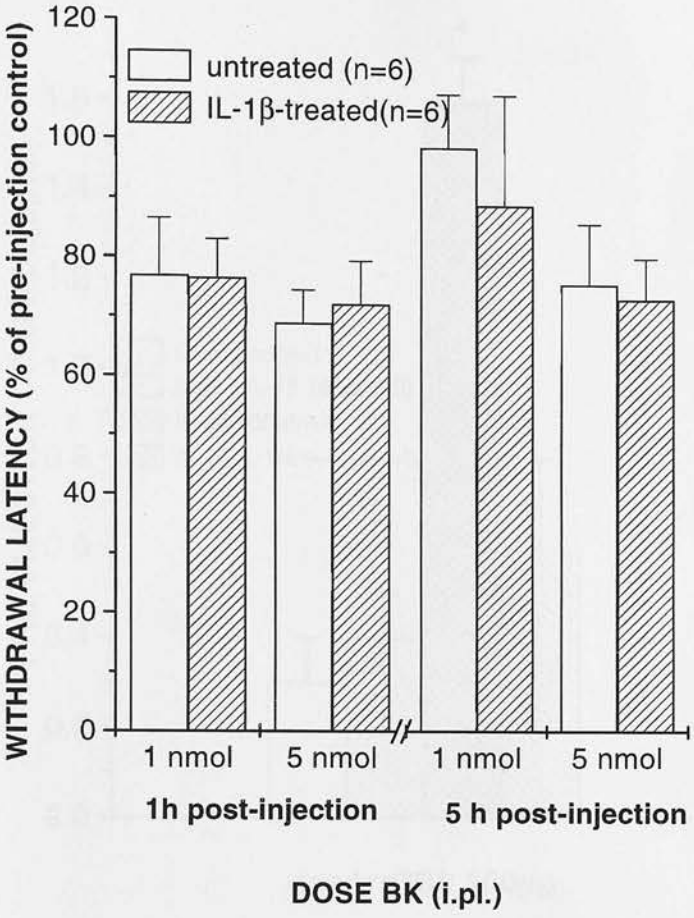


Figure 5.12

IL-1ß does not enhance the Bk-induced thermal hyperalgesia in the rat paw.

*The withdrawal latency following i.art. injection of Bk alone or co-injection with IL-1ß 0.1 u is shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

Kinin B_1-mediated effects

Intra-articular injection of IL-1β, but not boiled IL-1β, induced a kinin B_1 receptor-mediated increase in neural discharge. One hour following intra-articular injection of IL-1β 100 u, desArg^9-Bk increased neural discharge (1.6 ± 0.1 i.p.s.). There was no effect in untreated joints (0.3 ± 0.1 i.p.s.) or in those treated with boiled IL-1β (0.4 ± 0.1 i.p.s.). Co-injection of IL-1ra (0.1 μg) with IL-1β blocked the increase in neural discharge produced by desArg^9-Bk(Figure 5.13.).

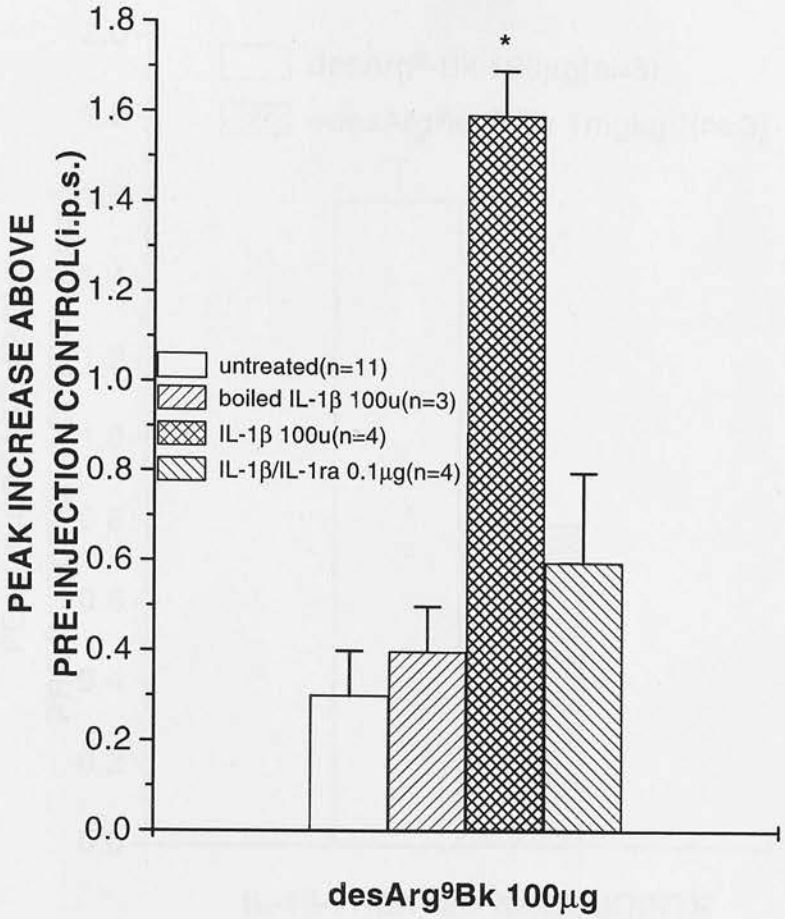


Figure 5.13

IL-1β-induces a desArg^9-Bk mediated increase in neural discharge in knee joint C-fibres, which is blocked by IL-1ra.

*Effects of desArg^9-Bk (i.a) in untreated, boiled IL-1β-treated, IL-1β-treated and IL-1β/IL-1ra-treated (i.art) joints are shown. All values are mean ± s.e.mean, * $p < 0.05$ compared to the effects in naïve joints, Mann Whitney U test.*

The desArg^9-Bk-induced increase in neural discharge was immediately reduced after i.a. injection of desArg^{9}leu^8-Bk (1 mgkg^{-1}) when the B_1 mediated increase in discharge decreased from 1.6 ± 0.1 i.p.s. to 0.8 ± 0.3 i.p.s. (Figure 5.14.).

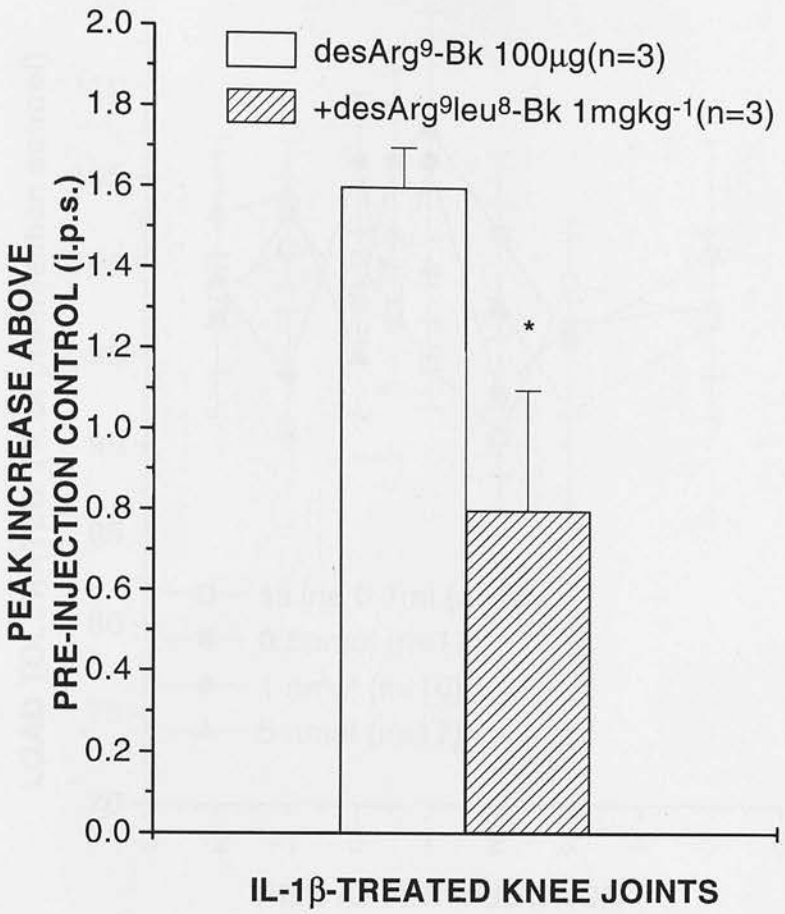


Figure 5.14

DesArg^9-Bk-induced increase in neural discharge in IL-1ß-treated knee joints is blocked by desArg^9 leu^8 Bk.

*Effects are shown for desArg^9-Bk 100 µg, i.a. before and following desArg^{9}leu8-Bk 1 mgkg^{-1} All values are mean ± s.e.mean, *p < 0.05 compared to desArg^9-Bk before antagonist, Mann Whitney U test.*

DesArg^9-Bk (0.5 - 5 nmoles, i.art.) had no effect on the load tolerated by the injected knee at any point throughout the time course (Figure 5.15.).

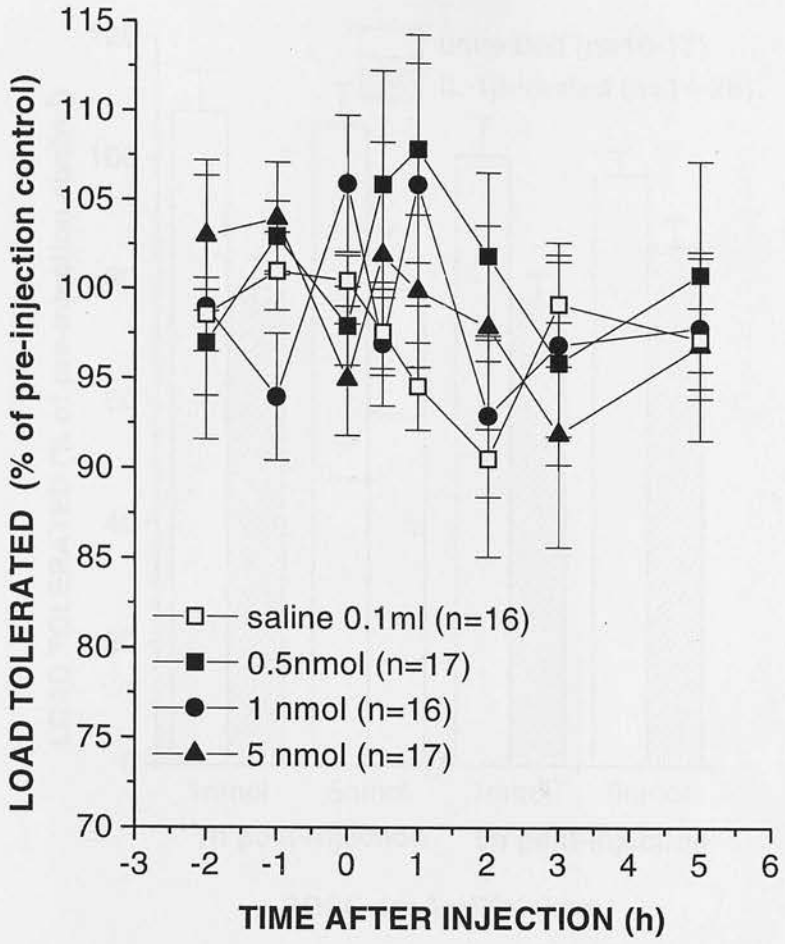


Figure 5.15

DesArg^9- Bk does not induce a mechanical hyperalgesia in naive knee joints.

*Effects of desArg^9- Bk(0.5 -5 nmol, i.art) are shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

However, when desArg^9-Bk (1 & 5 nmoles, i.art.) was co-injected with a sub-threshold dose of IL-1β (0.001 u) a reduction in load tolerated by the injected knee was observed. This was evident at both doses 1 h following injection, when the load tolerated decreased by 22 % (1nmole) and 22 % (5 nmoles). However, 5 h following injection a reduction was only observed at the lower dose, when the load tolerated was reduced by 23 % (Figure 5.16.).

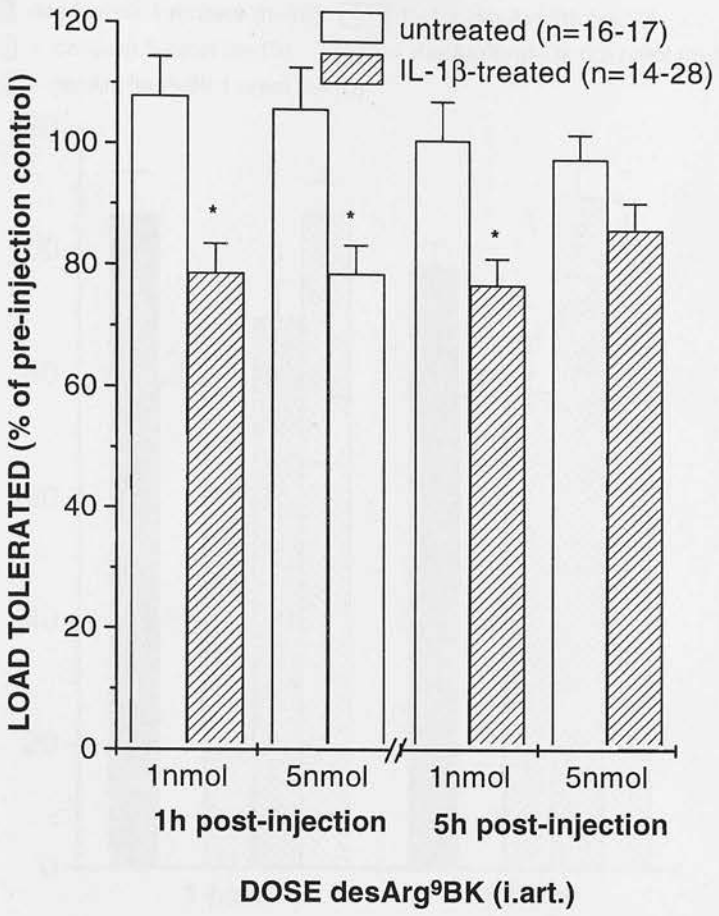


Figure 5.16

IL-1ß-induces a desArg^9- Bk mediated mechanical hyperalgesia in the rat knee joint.

*Effects of desArg^9-Bk alone and co-injection of IL-1ß 0.001 u with desArg^9-Bk on load tolerated are shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

The reduction in load tolerated induced by co-injection of desArg^9-Bk (1 nmole, i.art.) with IL-1β (0.001 u) was blocked when desArg^{9}leu^8-Bk (0.5 & 1 nmole, i.art.) was co-administered. This was evident 1 h and 5 h after injection. The B_2 antagonist, Icatibant (5 pmoles, i.art.) had no effect on the reduction in load tolerated by the injected knee (Figure 5.17.)

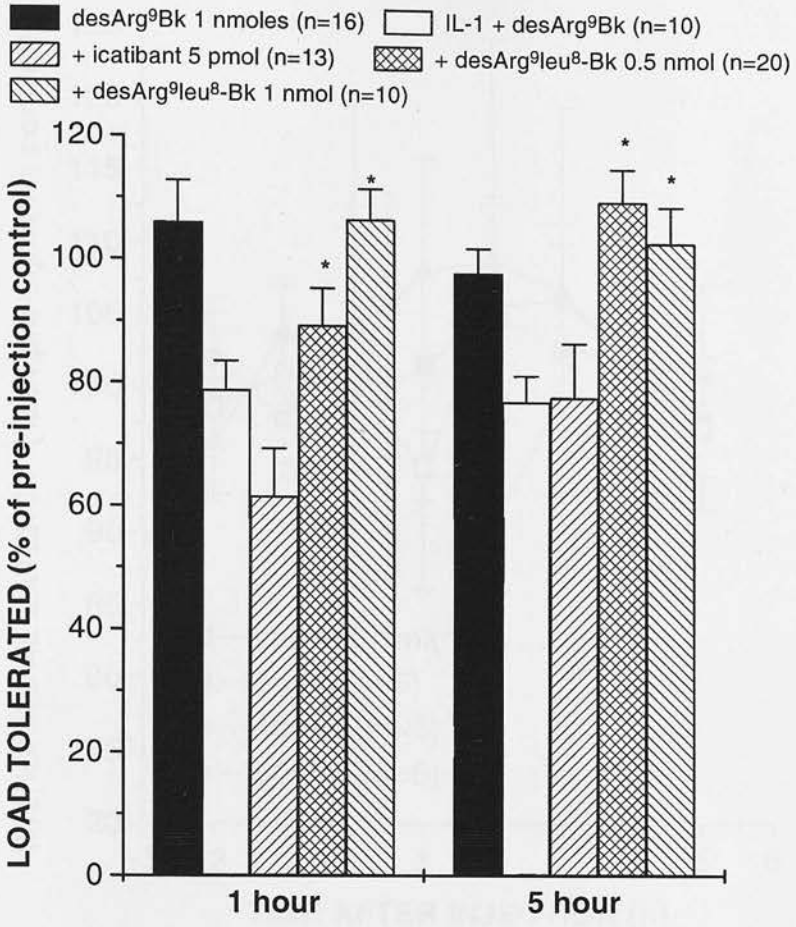


Figure 5.17

Kinin B_1, but not B_2 receptor antagonists block the mechanical hyperalgesia induced by desArg^9-Bk in the rat knee joint.

*Effects of desArg^9-Bk (1 nmole) with sub-threshold IL-1ß (0.001 u) alone and with icatibant and desArg^9 leu^8-Bk on load tolerated are shown. All values are mean ± s.e.mean. *p < 0.05, compared to open bars.*

DesArg^9-Bk (5 - 50 nmoles, i.pl.) had no effect on the withdrawal latency to focused radiant heat at any point throughout the time course (Figure 5.18.).

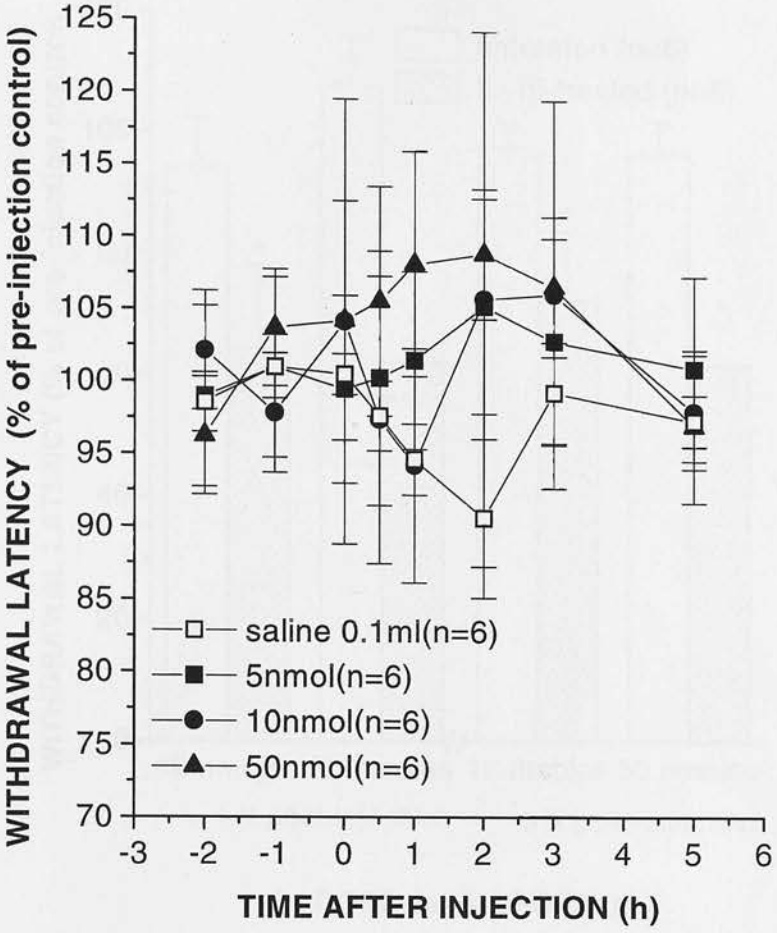


Figure 5.18

desArg^9-Bk does not induce a thermal hyperalgesia in naive rat paws.

*Effects of i.pl. desArg^9-Bk (5 - 50nmol) on withdrawal latency are shown. All values are expressed as means ± s.e.mean, * $p < 0.05$ compared to pre-injection control.*

When desArg^9-Bk (10 & 50 nmoles, i.pl.) was co-injected with a sub-threshold dose of IL-1β a reduction in withdrawal latency to a thermal stimulus was observed. This was evident at both doses 1 h following injection, when the withdrawal latency was reduced by 35.6 % (10 nmoles) and 37.5 % (50 nmoles) and also 5 h following injection when the latency was reduced by 27.2 % (10 nmoles) and 37.7 % (50 nmoles) (Figure 5.19.).

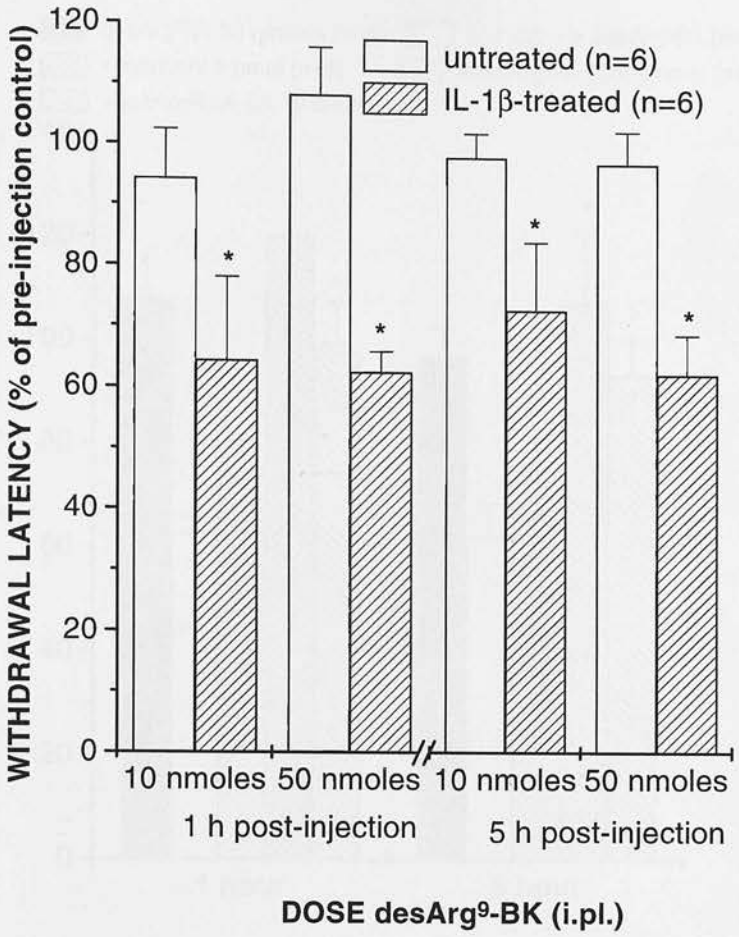


Figure 5.19

IL-1ß induces a desArg^9-Bk mediated thermal hyperalgesia in the rat paw.

*Effects of desArg^9-Bk alone and co-injection of IL-1ß 0.1 u with desArg^9-Bk on withdrawal latency are shown. All values are expressed as means ± s.e.mean, *p < 0.05 compared to pre-injection control.*

The reduction in withdrawal latency induced by co-injection of desArg^9-Bk (50 nmoles, i.pl.) with IL-1β (0.1 u) was blocked when co-administered with desArg^{9}leu^8-Bk (1 & 10 nmole, i.pl.). This was evident 1 h and 5 h after injection. The B_2 antagonist, icatibant (5 pmoles, i.art.) had no effect on the reduction in withdrawal latency in response to thermal stimulus of the injected paw (Figure 5.20.)

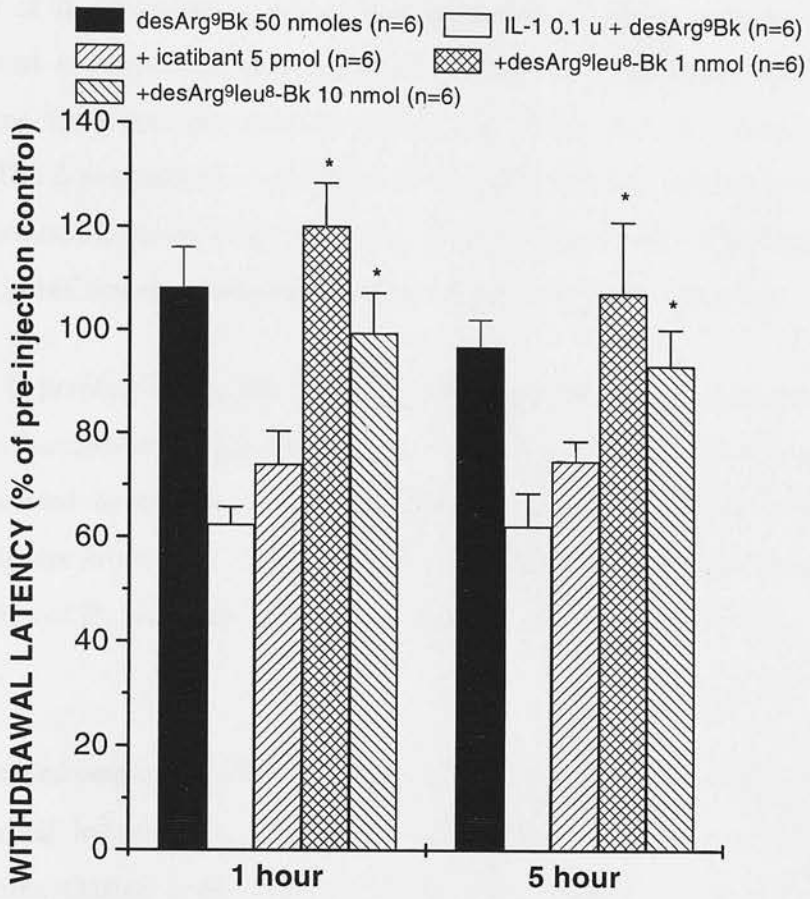


Figure 5.20

Kinin B_1, but not B_2 receptor antagonists block the thermal hyperalgesia induced by desArg^9-Bk in the rat paw.

*Effects of desArg^9-Bk (50 nmole) with sub-threshold IL-1β (0.1 u) alone and with icatibant and desArg^9 leu^8-Bk on withdrawal latency are shown. All values are mean ± s.e.mean. *p < 0.05, compared to open bars*

5.4 Discussion.

These results provide evidence that local injection of IL-1β increases C-fibre activity *in vivo* and can induce both mechanical and thermal hyperalgesia. They also show that IL-1β can sensitise nociceptors to Bk and provide further evidence for a role for IL-1β in the induction of a B_1 mediated hyperalgesia in inflamed knee joints.

Local injection of IL-1β increased neural discharge from C-fibres innervating the rat knee joint and induced a mechanical and thermal hyperalgesia. The doses used to induce an increase in firing from nociceptors were higher than those used to induce a behavioural hyperalgesia. The doses used however, were comparable to those used to activate cutaneous C-fibres in the saphenous nerve (Fukuoka *et al.*, 1994). Fukuoka also reported a similar delay to onset of action and duration following local administration of the cytokine.

A behavioural hyperalgesia has been observed with similar doses in a variety of animal models. In the Randall-Selitto paw-withdrawal test, IL-1β induced mechanical hyperalgesia which was mediated by release of prostaglandins (Cunha *et al.*, 1992). Mechanical and thermal hyperalgesia following inflammation or IL-1β administration has also been shown to involve kinin B_1 and B_2 receptors as well as prostaglandins (Davis & Perkins, 1994; Perkins & Kelly, 1994).

IL-1β also increased responses to Bk in both neural recordings and mechanical hyperalgesia, but not in thermal hyperalgesia. IL-1β can upregulate kinin B_2 receptors and enhance responses to kinins (Galizzi *et al.*, 1994; Lerner & Modeer, 1991). These enhanced responses may be due to release of prostaglandins which have been shown to sensitise nociceptors to Bk (Weinreich, 1986; Birrell *et al.*, 1991). Such an effect may be further enhanced as IL-1β can act in synergy with Bk to release prostanoids from cells including fibrobasts (Sundqvist & Lerner, 1996; Lerner & Modeer, 1991) and synovial cells(O'Neil & Lewis, 1989). The enhanced responsiveness to Bk in mechanical hyperalgesia is observed 1 hour and 5 hours after injection with the lower dose of Bk, however this is only observed at the early time point with the higher dose of Bk. It could be that 5 hours after injection the higher dose of Bk could synergise sufficiently with IL-1β to release mediators which regulate the inflammatory process or associated hyperalgesia. In previous chapters it was observed that in both inflamed ankle joints and knee joints the increase in neural discharge induced by Bk had a tendency to be lower than that observed in normal joints. There could be several reasons for this. Both Bk and IL-1β can lead to formation of nitric oxide which the present studies have shown to reduce Bk responses in arthritic ankle joints and has been shown to be involved in desensitisation of the Bk receptor. IL-1β can also release several anti-inflammatory cytokines

including, IL-4, IL-10 and IL-13 which have the ability to modulate the effects of IL-1β.

It could also be that Bk is degraded more rapidly in inflammation. Levels of kininases have been shown to be elevated in inflammatory conditions, which may be the case at the later period of the experiment. Somewhat surprising results were obtained in the thermal experiments as no increase in Bk responsiveness was observed when administered with IL-1β. As yet there is no explanation for this, but it may be that there is a difference between thermal and mechanical stimulus or there is a difference between the paw and the knee joint, for example, Bk may be broken down more rapidly within the paw. To investigate this a mechanical stimulus could be used in the paw and compared to the results obtained using the thermal stimulus.

The IL-1β-induced kinin B_1-mediated increase in neural discharge, mechanical hyperalgesia and thermal hyperalgesia observed here is consistent with previous studies relating this cytokine to B_1 kinin receptor induction. IL-1β has been shown to induce B_1 mediated responses in blood vessels, an action which appears to involve induction of the receptor as it can be blocked by cyclohexamide, a protein synthesis inhibitor. It has also been shown to induce a B_1 mediated mechanical and thermal hyperalgesia in behavioural models (Davis & Perkins, 1994; Perkins & Kelly, 1994). As discussed previously, there is, as yet, no evidence of B_1 receptors on sensory neurons, and the delay to onset of action of desArg^9-Bk suggests that the B_1 mediated increase in neural discharge and behavioural hyperalgesia is indirect, possibly mediated through release of inflammatory mediators from non-neuronal cells such as macrophages, fibroblasts, synovial cells, endothelial cells and smooth muscle cells (Tiffany & Burch, 1989; Lerner & Modeer, 1991; Bathon *et al.*, 1989; Cahill *et al.*, 1988; Galizzi *et al.*, 1994). IL-1β can synergise with kinin B_1 agonists to release prostaglandins from fibroblasts (Lerner & Modeer, 1991). In the mechanical hyperalgesia model although IL-1β induced a B_1 mediated hyperalgesia with the lower dose at both the early and late time point, the higher dose was only hyperalgesic at the 1 hour time point. The reasons for this may be the same as discussed with Bk.

These results provide evidence for a role of IL-1β in hyperalgesia. They also indicate a role for IL-1β in increasing the responses to desArg^9-Bk and Bk. The results also suggest there

may be a difference between acute and a more persistent inflammation as with higher doses of kinins no enhancement of response was observed at the later time points or indeed in arthritic knee joints - Bk has a tendency to induce a smaller increase in neural discharge than observed in normal joints. This may be due to an autoregulation of the inflammatory process with anti-inflammatory or anti-nociceptive modulation occurring.

6. THE ROLE OF PROSTAGLANDINS IN IL-1β MEDIATED HYPERALGESIA AND MODULATION OF KININ ACTIVITY: ELECTROPHYSIOLOGICAL AND BEHAVIOURAL STUDIES.

6.1 Introduction.

The pro-inflammatory cytokine, IL-1β, has a multitude of pro-inflammatory actions. When IL-1β binds to its receptor, the IL-1RI receptor, it releases arachidonic acid via activation of phospholipase A_2 (Gronich *et al.*, 1994) with subsequent production of prostaglandins from a variety of cells including macrophages, endothelial cells, fibroblasts and synovial cells (Billingham, 1987). As well as producing prostanoids directly, IL-1β also upregulates the inducible cyclooxygenase enzyme (COX-2) in human synovial cells (Bathon *et al.*, 1994) resulting in increased prostaglandin synthesis. The role of prostaglandins in IL-1β mediated hyperalgesia is somewhat confusing as indomethacin has been shown to reduce the hyperalgesic effects in some studies (Cunha *et al.*, 1992; Davis & Perkins, 1994), but has no effect in others (Follenfant *et al.*, 1989). However, studies in the cat knee joint have shown that both PGE_2 and PGI_2 excite and sensitise a large proportion of C-fibres (Schepelmann *et al.*, 1992) suggesting that prostaglandins have the ability to produce hyperalgesia.

Bk acting via B_2 receptors on non-neuronal cells including fibroblasts, synovial cells and smooth muscle cells can also activate membrane phospholipase A_2 resulting in the production of prostanoids (Bareis *et al.*, 1983; Burch & Axelrod, 1987; Bathon *et al.*, 1989; Galizzi *et al.*, 1994). Prostaglandins can increase pain to injection of Bk in human skin and veins(Kindgen-Milles, 1995) and can sensitise sensory nerves to Bk in the cat knee joint (Schepelmann *et al.*, 1992) and rat ankle joint (Birrell *et al.*, 1991). The mechanism which probably underlies this sensitisation is as follows: prostaglandins acting on prostanoid receptors can then activate adenylate cyclase, thereby raising levels of cAMP which in turn inhibits Ca^{++}-dependent K^+ permeability. This reduces the post-spike hyperpolarisation which is K+ dependent and can lead to an increase in excitability of the cell (Weinreich, 1986).

As with the B_2 receptor, activation of the B_1 receptor activates membrane bound phospholipase A_2 with subsequent production of prostanoids from endothelial cells, smooth

muscle cells, fibroblasts and macrophages (D'Orleans-Juste *et al.*, 1989; Galizzi *et al.*, 1994; Marceau & Tremblay, 1986; Tiffany & Burch, 1989; Marceau *et al.*, 1995). IL-1β can also synergise with B_1 receptor agonists on fibroblasts to induce production of prostaglandins (Lerner & Modeer, 1991).

These experiments have, therefore, investigated the role of prostaglandins, using an inhibitor of COX, indomethacin, in IL-1β-induced increase in neural discharge The role of prostanoids in the modulation of kinin B_1 and B_2-mediated effects, by IL-1β was also investigated.

6.2 Methods.

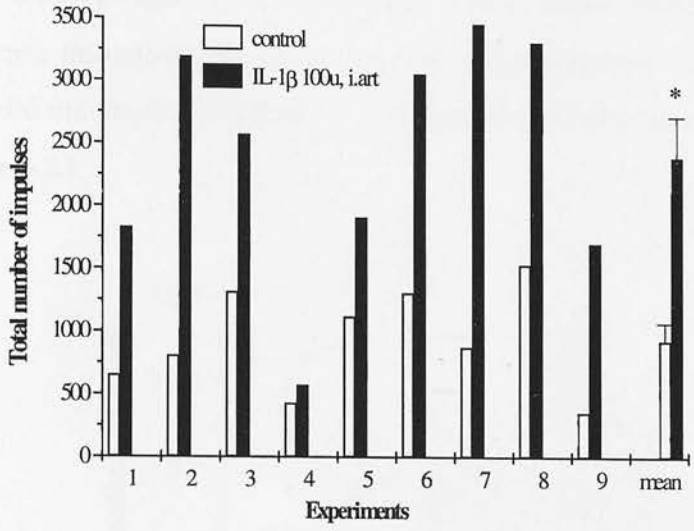
These were as described in sections 2.3. and 5.2. (neural experiments) and 2.4 (behavioural experiments). In neural experiments indomethacin was administered systemically (i.a.) 30 minutes before i.art. injection of the algesic agents into knee joints. In the behavioural experiments indomethacin was injected subcutaneously with an injection volume of 0.1ml in all cases.

6.3 Results.

In the behavioural experiments discussed in this section, the pre-injection control values were as follows. The load tolerated before i.art. injection was 93.2 ± 1.1g (range: 70 - 146g, n = 161). The withdrawal latency in the thermal hyperalgesia model before i.pl. injection was 10.1 ± 0.3 s (range: 6.7 - 14.8 s, n = 47).

Effects of indomethacin on IL-1β-induced effects on the neural discharge of C-fibres and on behavioural hyperalgesia.

When indomethacin (1 $mgkg^{-1}$, i.a.) was administered 30 min prior to IL-1β (100 u, i.art.) the IL-1β-induced increase in neural discharge was blocked (Figure 6.1.).



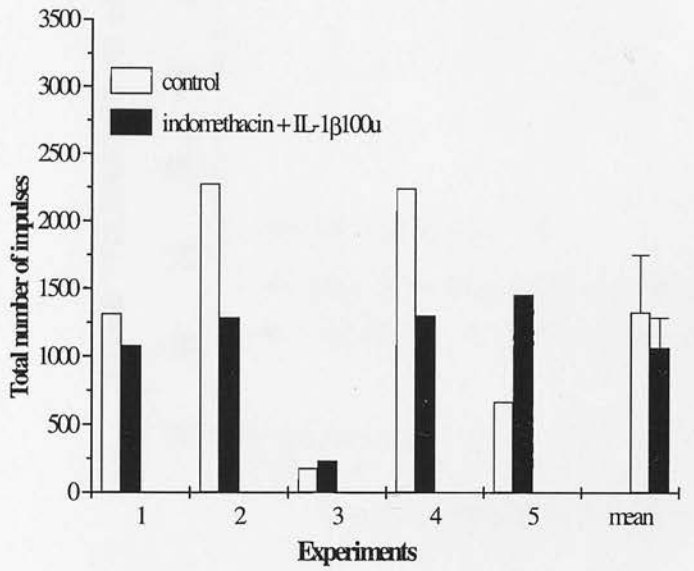


Figure 6.1

Indomethacin blocks IL-1β–induced increase in neural discharge.

*(top graph) Effects of IL-1β 100 u alone and (lower graph) following pre-treatment (30 min) with indomethacin 1 mgkg^{-1}, i.a. on neural discharge are shown. Bargraphs showindividual expetiments and their respective mean ± s.e.m., * $p < 0.05$ compared to indomethacin treated, Wilcoxan matched pairs test.*

In the behavioural hyperalgesia experiments, pre-treatment (30 min.) with indomethacin (1mgkg^{-1}, s.c.) blocked the IL-1β-induced (10 u, i.art.) reduction in load tolerated by the injected knee throughout the time course of the experiment. Boiled IL-1β had no effect by itself nor did indomethacin influence the tolerated load when given 30 min. prior to boiled IL-1β (Figure 6.2.).

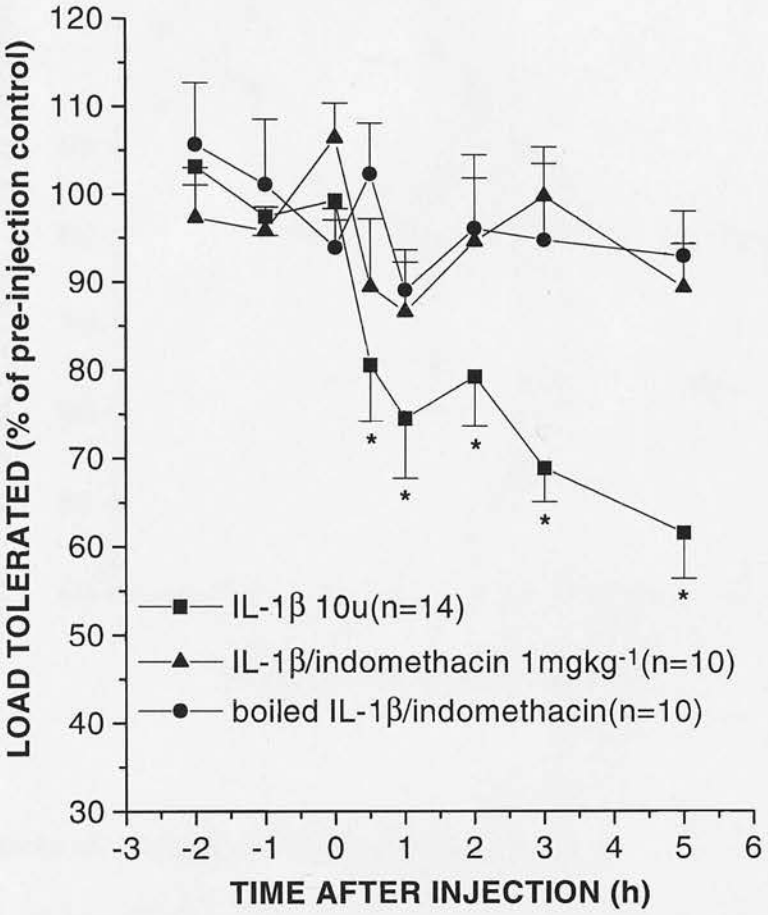


Figure 6.2

Indomethacin blocks IL-1β–induced mechanical hyperalgesia.

*Effects of IL-1β 10 u alone, following pre-treatment (30 min, s.c.) with indomethacin and indomethacoin pre-treatment followed by boiled IL-1β are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to pre-injection control.*

Pre-treatment (30 min.)with indomethacin (1 mgkg^{-1}, s.c.) also blocked the IL-1β (10 u, i.pl.)-induced reduction in withdrawal latency to a focused radiant heat throughout the time course

of the experiment (Figure 6.3.).

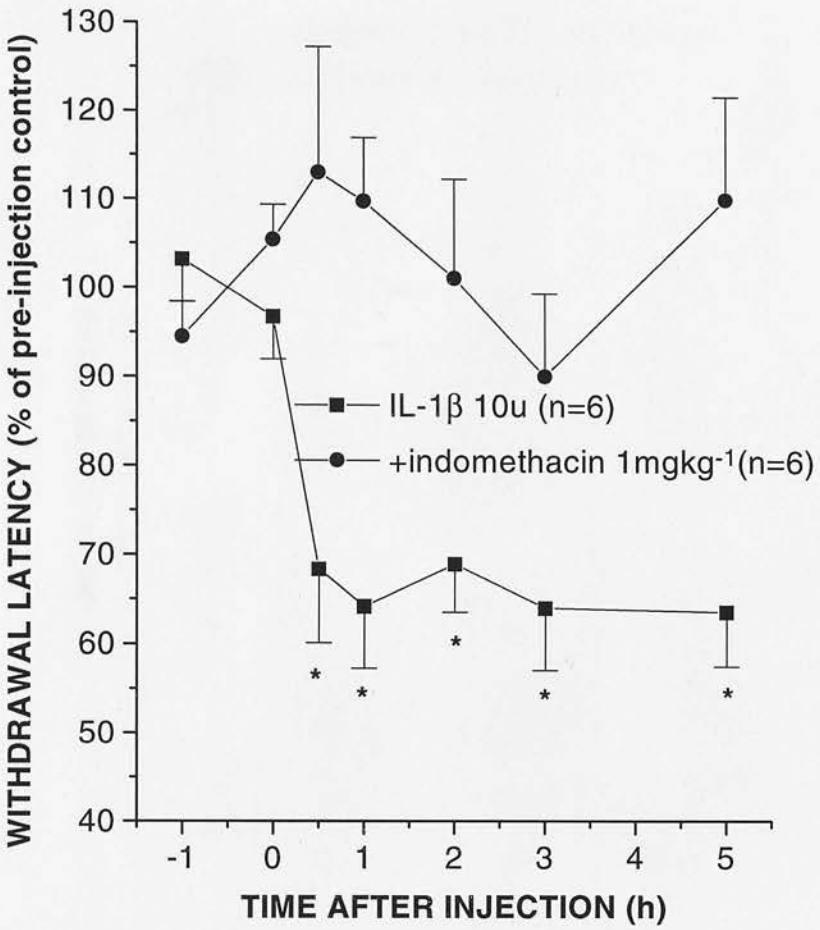


Figure 6.3

Indomethacin blocks IL-1β–induced thermal hyperalgesia.

*Effects of i.pl. IL-1β 10 u alone and following pre-treatment (30 min, s.c.) with indomethacin on withdrawal latency are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to pre-injection control.*

Effects of indomethacin on IL-1β-induced effects on Bk-induced activation of C-fibres and Bk-induced mechanical hyperalgesia.

The enhancement of C-fibre activity by Bk in joints pretreated with IL-1β was prevented by pre-treatment (30 min.) with indomethacin (1 $mgkg^{-1}$, i.a.). The increase in neural discharge observed in indomethacin pre-treated rats was no different to that observed in untreated knee

joints (Figure 6.4.).

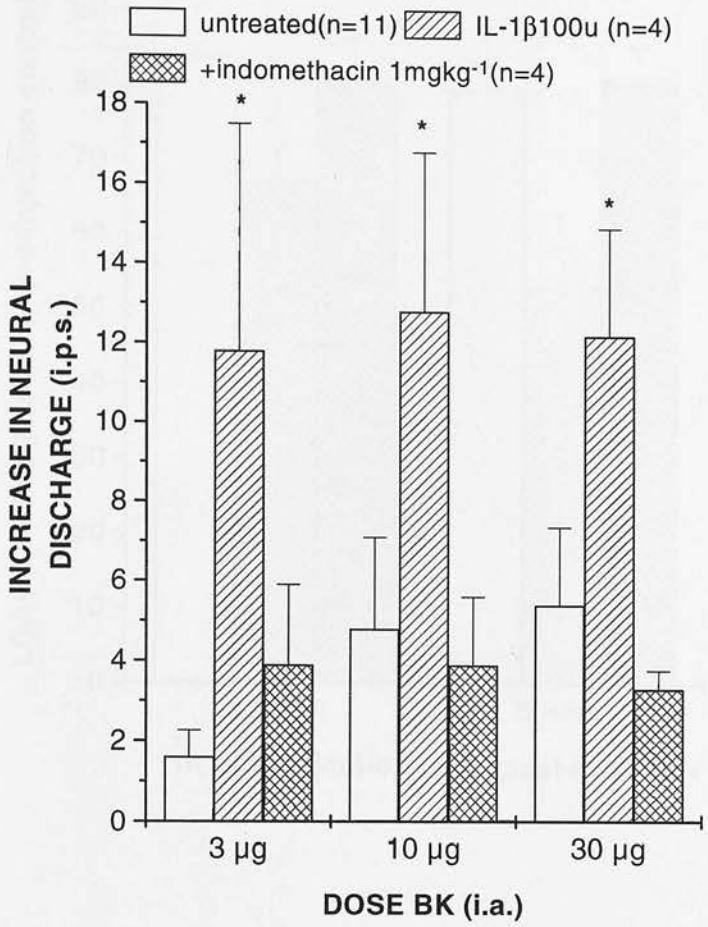


Figure 6.4

Indomethacin blocks IL-1β–induced increase in C-fibre responsiveness to Bk.

*Effects of i.a. Bk in untreated knee joints IL-1β-treated joints: 100 u, i.art. and following pre-treatment (30 min, i.a.) with indomethacin 1 mgkg^{-1}, i.a. are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to untreated knee joint control.*

The enhanced reduction in load tolerated observed 1 and 5 h following co-injection (i.art.) of a sub-threshold dose of IL-1β (0.001 u) and Bk (0.1 nmol) was blocked by pre-treatment (30 min.) with indomethacin (1 mgkg^{-1})[Figure 6.5.].

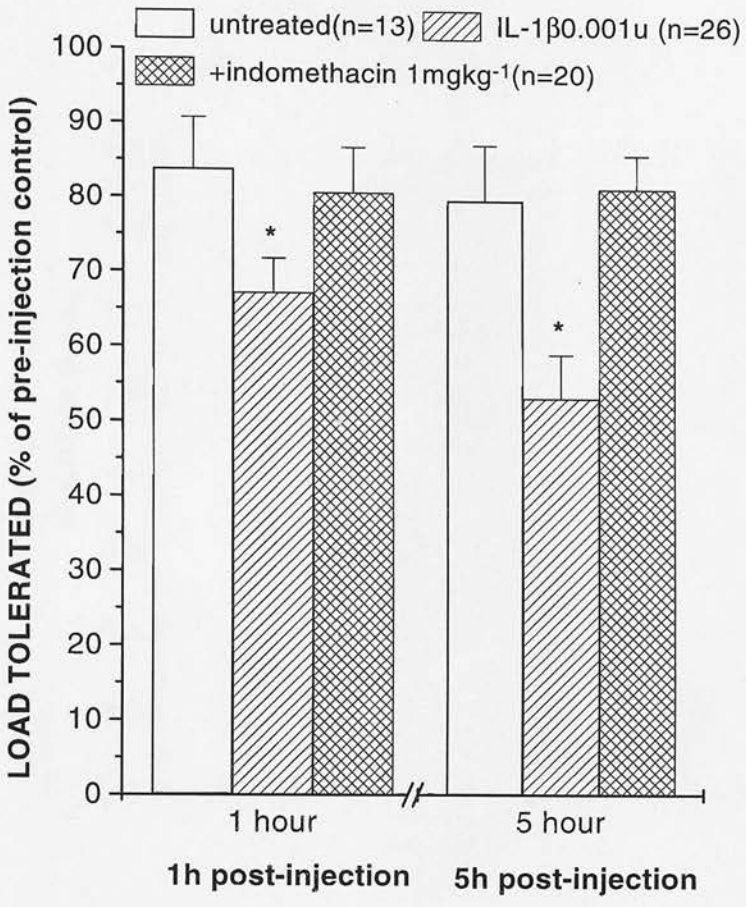


Figure 6.5

Effect of indomethacin on i.art. IL-1β–induced enhanced mechanical hyperalgesia induced by Bk.

*Effects of Bk 0.1 nmol, i.art. alone, and co-injected with sub-threshold IL-1β and following pre-treatment (30 min, s.c.) with indomethacin on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to open bars.*

Effects of indomethacin on IL-1β-induced responses of C-fibres to desArg^9-Bk-induced on desArg^9-Bk-induced mechanical and thermal hyperalgesia.

Pre-treatment (30 min.) with indomethacin (1 mgkg^{-1}, i.a.) also blocked the enhanced responsiveness of C-fibres to i.a. desArg^9-Bk in IL-1β-treated knee joints. There were no effects of desArg^9-Bk in either untreated knee joints or in those rats which had been pre-treated with indomethacin (Figure 6.6.).

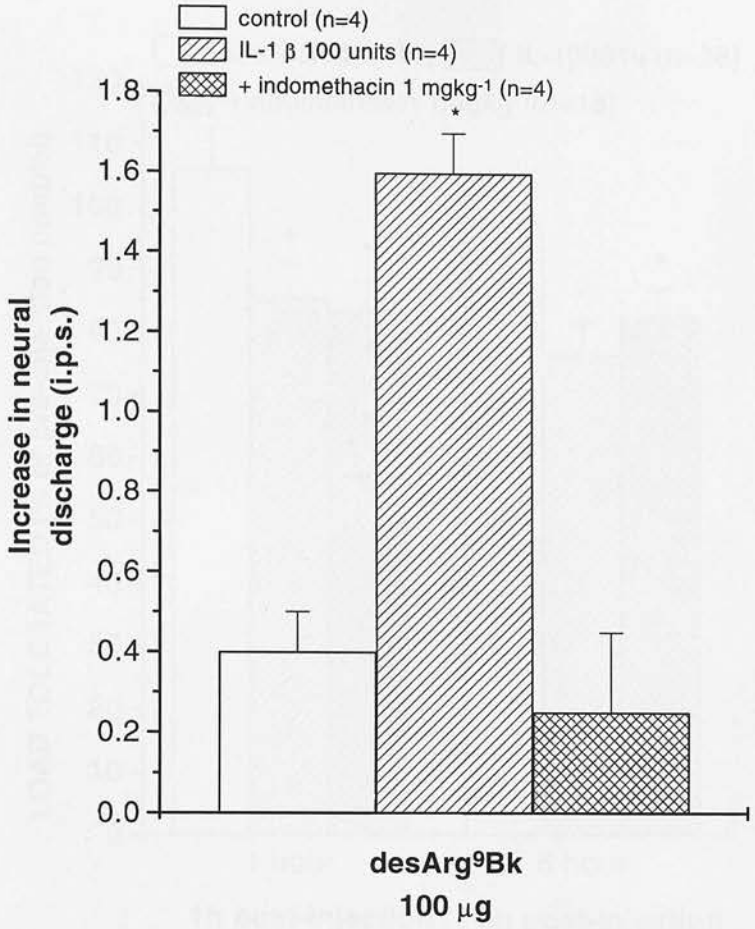


Figure 6.6

Indomethacin blocks IL-1β–induced desArg^9-Bk-mediated increase in neural discharge.

*Effects of i.a. desArg^9-Bk in untreated knee joints IL-1β-treated joints: 100 u, i.art. and following pre-treatment (30 min, i.a.) with indomethacin 1 mgkg^{-1}, i.a. are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to untreated knee joint control.*

The reduction in load tolerated 1 and 5 h following co-injection (i.art.) of a sub-threshold dose of IL-1β (0.001 u) and desArg^9-Bk (1 nmol) was not blocked by pre-treatment (30 min.) with indomethacin (1 mgkg^{-1}) [Figure 6.7.].

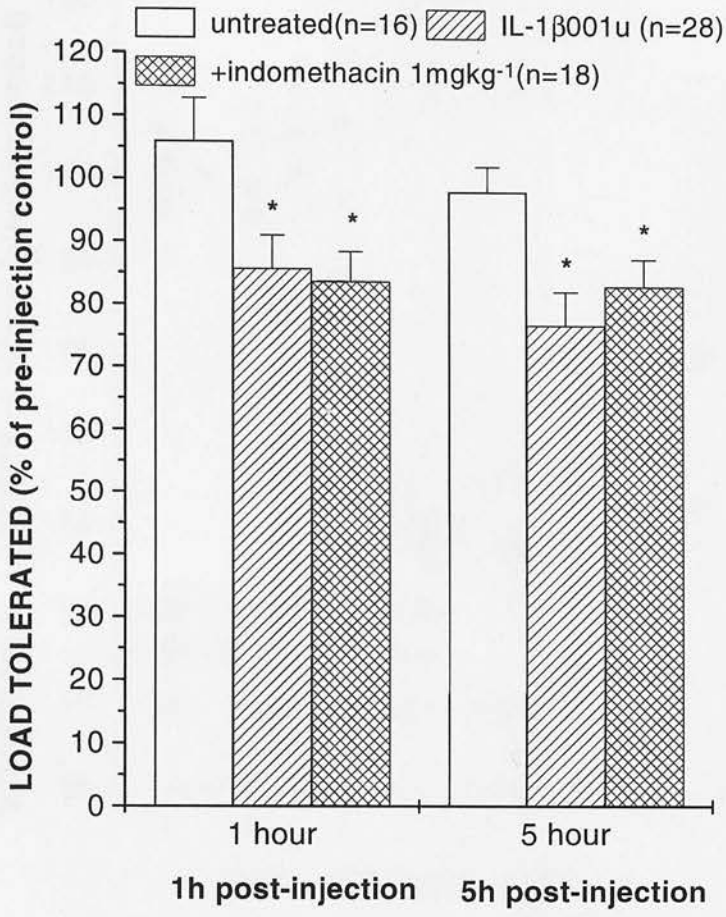


Figure 6.7

Indomethacin has no effect on IL-1β–induced desArg^9-Bk-mediated mechanical hyperalgesia.

*desArg^9-Bk 1 nmol, i.art. (open bar, n = 16) alone, and co-injected with sub-threshold IL-1β, 0.001 u (hatched bars, n = 28) and IL-1β with Bk following pre-treatment (30 min) with indomethacin 1 mgkg^{-1}, s.c. (cross hatched bars, n = 18). All values are mean ± s.e.m., * p < 0.05 compared to open bars.*

The reduction in withdrawal latency to a thermal stimulus following a co-injection (i.pl.) of a sub-threshold dose of IL-1β (0.1 u) and desArg^9-Bk (50 nmol) was prevented by pre-treatment (30 min) with indomethacin (1 mgkg^{-1}, s.c.) but only for 2h following injection of desArg^9-Bk (50 nmol) and IL-1β (0.1 u)[Figure 6.8.].

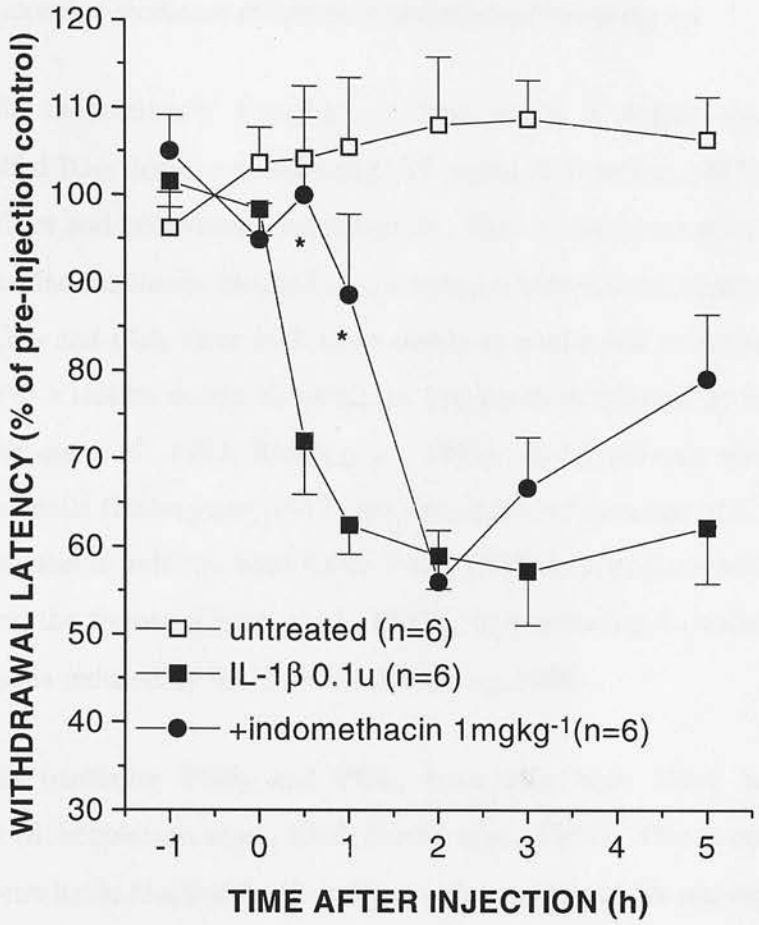


Figure 6.8

Indomethacin only blocks IL-1β–induced desArg^9-Bk-mediated thermal hyperalgesia 60 minutes after injection of the B_1 antagonist.

*Effects of desArg^9-Bk 50 nmol, i.pl. alone, and co-injected with sub-threshold IL-1β, 0.1 u and following pre-treatment (30 min, s.c.) with indomethacin on withdrawal latency are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to closed square.*

6.4 Discussion.

These results provide evidence that IL-1β-induced increase in neural discharge from C-fibres *in-vivo* and behavioural hyperalgesia is mediated via release of prostanoids. The release of prostanoids is also responsible for the IL-1β-induced enhancement of Bk-induced activation of C-fibres and mechanical hyperalgesia. However, prostanoids only seem to be involved in the IL-1β-induced kinin B_1 receptor-mediated increase in neural discharge, but not the IL-1β-

induced kinin B_1 receptor-mediated mechanical and thermal hyperalgesia.

Pre-treatment with indomethacin 1 $mgkg^{-1}$, a dose which is within the same range as previously published ID_{50} doses, e.g, $0.8mgkg^{-1}$ (Tonussi & Ferreira, 1992). IL-1β-induced activation of C-fibres and behavioural hyperalgesia. This is consistent with previous studies which showed that indomethacin blocked IL-1β-induced behavioural hyperalgesia (Cunha *et al.*, 1992) and PGE_2 and PGI_2 have both been shown to excite and sensitise C-fibres. Both prostaglandins have a similar action although the sensitisation induced by PGI_2 has a longer duration (Schepelmann *et al.*, 1992; Birrell *et al.*, 1991). IL-1β not only releases prostanoids from inflammatory cells (Billingham, 1987) but also induces formation of COX-2 (Bathon *et al.*, 1994). Indomethacin inhibits both COX-1 and COX-2 (approximately 1.7 times more potent at inhibiting the former (Ogino *et al.,* 1997)), thus reducing formation of prostanoids and thus hyperalgesia induced by IL-1β (Vane & Botting, 1996).

Prostaglandins, in particular PGE_2 and PGI_2, have also been show to sensitise joint nociceptors to Bk (Scheppleman *et al.*, 1992; Birrell *et al.*, 1991). This is consistent with our findings that indomethacin blocked IL-1β-induced enhancement of Bk responses on knee joint C-fibres and IL-1β-induced enhancement of Bk mediated mechanical hyperalgesia. Prostanoid EP_2 receptors have been shown to be involved in the sensitisation of nociceptors to heat (Kumazawa *et al.*, 1994) whereas EP_3 receptors have been shown to sensitise nociceptors to Bk (Kumazawa *et al.*, 1993). The mechanism of sensitisation is probably as follows. When prostanoids bind to their receptors this activates adenylate cyclase resulting in production of cAMP and activation of protein kinase A and inhibition of Ca^{++}-dependent K^+ ion channel (Wang *et al.*, 1996).

Indomethacin blocks kinin B_1 mediated increase in neural discharge induced by IL-1β but is ineffective in IL-1β-induced kinin B_1 receptor mediated mechanical hyperalgesia and only effective for up to 2 hours in kinin B_1 mediated thermal hyperalgesia. This suggests the increase in neural discharge and the behavioural hyperalgesia induced by B_1 receptor activation have different underlying mechanisms. One possibility are leukotrienes, products of the lipoxygenase pathway. Kinin B_1 agonists have been shown to release LTB_4 from macrophages (Sato *et al.*, 1996) and LTB_4 has been shown to sensitise nociceptors to

mechanical and thermal stimulus (Martin *et al.*, 1987).

These results show that prostaglandins are involved in the IL-1β-induced increase in neural discharge from C-fibres and behavioural hyperalgesia and IL-1β-induced enhancement of kinin mediated increase in neural discharge and B_2-mediated mechanical hyperalgesia. This, however, is not the only mechanism as indomethacin has no effect on IL-1β-induced kinin B_1 mediated behavioural hyperalgesia suggesting that more than one pathway is involved.

7. THE MODULATORY ROLE OF IL-4 AND IL-10 ON IL-1β MEDIATED HYPERALGESIA AND MODULATION OF KININ ACTIVITY IN BEHAVIOURAL STUDIES.

7.1 Introduction.

The previous studies have explored the modulation of both C-fibre activity and behavioural hyperalgesia in inflamed joints. In arthritic knee joints Bk-induced increase in neural discharge had a tendency to be lower than in normal joints. Also, in behavioural experiments, regarding the modulation of kinin activity by IL-1β, some unusual results were observed. IL-1β did not enhance hyperalgesia, when higher doses of kinins were used, at later time points during experiments.

Although most cytokines studied to date are primarily thought of as pro-inflammatory, several cytokines have been shown to possess anti-inflammatory properties (Burger & Dayer, 1995). Their actions appear to involve the regulation or modulation of the actions of inflammatory cytokines, particularly IL-1β. Three such cytokines; IL-4, IL-10 and IL-13, have been shown to be released from activated T-cells, monocytes and mast cells (MacNeil *et al.*, 1990; Spits *et al.*, 1992; de Waal Malefyt *et al.*, 1991; Burd *et al.*, 1995), all cell types prevalent in inflammation..

IL-4 and IL-13 act on receptors which bear similarities to each other (Vita *et al.*, 1995; Smerz-Bertling & Duschl, 1995). It is not surprising then, that they have similar actions; both inhibit production of inflammatory cytokines including IL-1β, from monocytes (Essner *et al.*, 1989; te Velde *et al.*, 1990; Minty *et al.*, 1993), upregulate the production and relase of IL-1ra (Vannier *et al.*, 1992; Vannier *et al.*, 1996) and IL-1RII (Colotta *et al.*, 1993; Colotta *et al.*, 1996; Girard *et al.*, 1996). The actions by IL-10 are somewhat similar to those of IL-4; including downregulation of IL-1 production and upregulation of IL-1ra production (Jenkins *et al.*, 1994). However, IL-4 and IL-10 appear to differ in some ways with respect to regulation of inflammatory mediators. IL-4, but not IL-10, reduces PGE_2 release from synoviocytes (Dechanet *et al.*, 1995) and TNFα release from monocytes (Joyce *et al.*, 1994).

These studies investigated whether these putative 'anti-inflammatory' cytokines could modulate IL-1β induced mechanical and thermal hyperalgesia as well as the IL-1β-induced enhancement of kinin activity. To accomplish this we made use of two such anti-inflammatory cytokines. Bearing in mind the similarities between IL-4 and IL-13 and the differences observed between IL-4 and IL-10, it was decided to look at the effects of the latter two.

7.2 Methods.

As described in section 2.4. In these experiments either IL-4 or IL-10 was injected concomitantly with: IL-1β; IL-1β and desArg^9-Bk; or IL-1β and Bk.

7.3 Results.

In the behavioural experiments discussed in this section, the pre-injection control values were as follows. The load tolerated before i.art. injection of IL-1β, IL-1β and desArg^9-Bk, or IL-1β and Bk was 92.7 ± 0.8 g (range: 70 - 146g, n = 260). The withdrawal latency in the thermal hyperalgesia model before i.pl. injection was 10.3 ± 0.3 s (range: 6.5 - 15 s, n = 76).

Effects of IL-4 and IL-10 on IL-1β-induced effects in behavioural hyperalgesia.

IL-4 (1 & 10 ng, i.art.) co-injected with IL-1β alleviated the reduction in load tolerated by the injected knee seen with IL-1 β, although this was not dose-dependent at the two doses used here. IL-4 co-injected with boiled IL-1β had no effect on the load tolerated by the injected knee (Figure 7.1.).

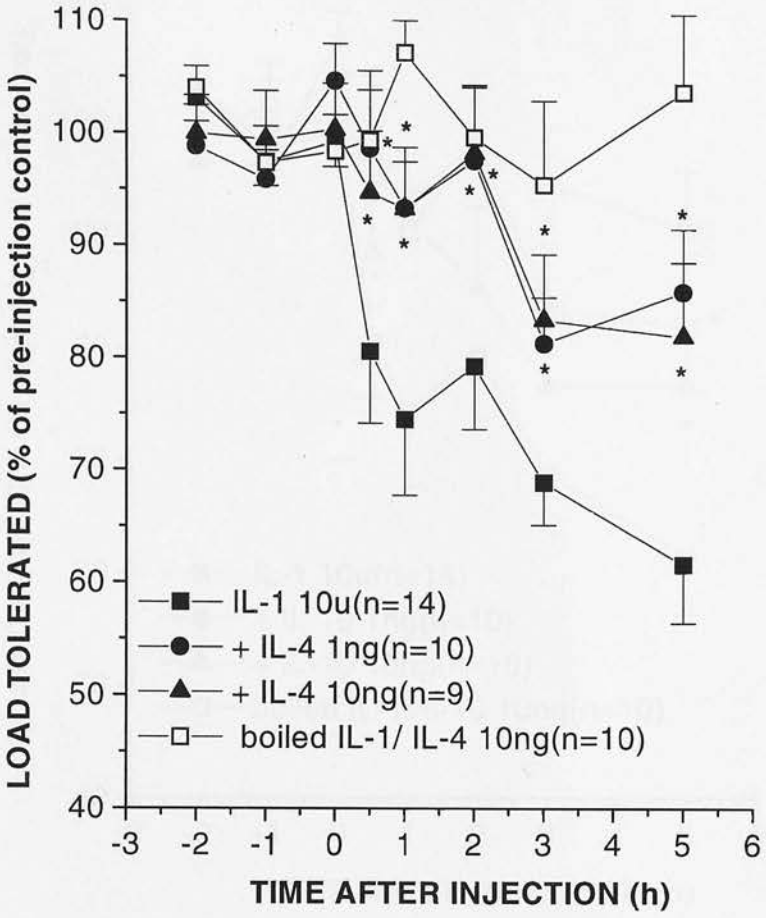


Figure 7.1

IL-4 reduces IL-1β–induced mechanical hyperalgesia.

*Effects of i.art. IL-1β alone, co-injected with IL-4 1 ng and 10ng and IL-4 10 ng co-injected with boiled IL-1β on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to IL-1 β alone.*

IL-10 (1 & 10 ng, i.art.) co-injected with IL-1β also prevented the reduction in load tolerated by the injected knee following IL-1 β and, as with IL-4, this was not dose-dependent. IL-10 co-injected with boiled IL-1β had no effect on the load tolerated by the injected knee (Figure 7.2.).

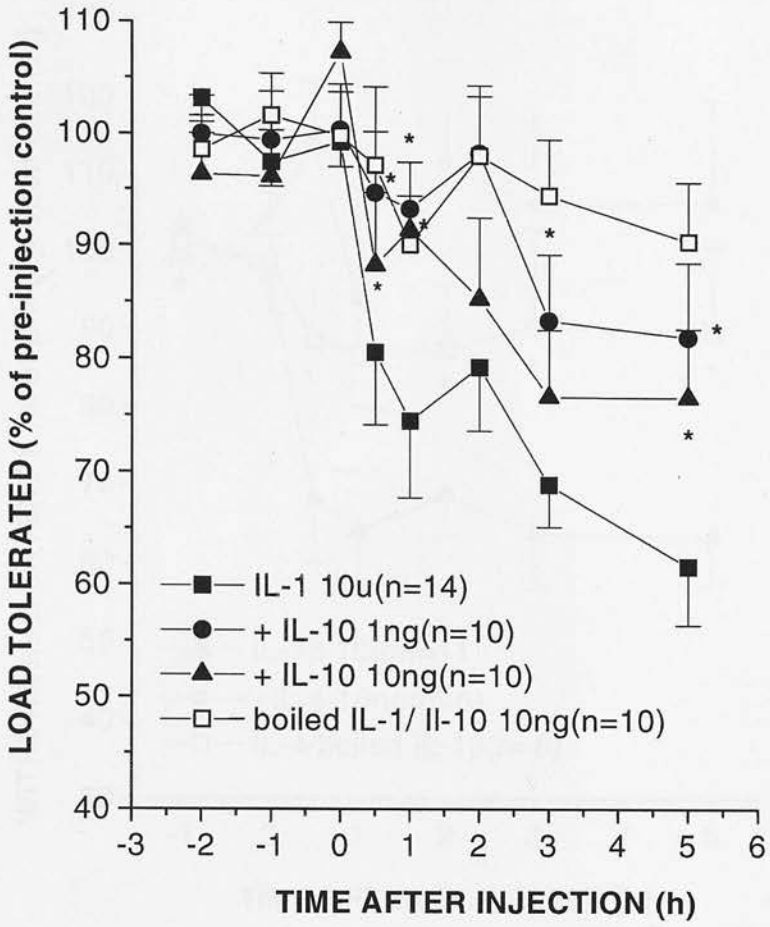


Figure 7.2

IL-10 reduces IL-1β–induced mechanical hyperalgesia.

*Effects of i.art. IL-1β alone, co-injected with IL-10 1 ng and 10 ng and IL-10 10 ng co-injected with boiled IL-1β on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to IL-1 β alone.*

The reduction in withdrawal latency induced by i.pl. IL-1β (10 u) to a thermal stimulus was prevented by co-injection with IL-4 (10 ng). IL-4 had no significant effect on paw withdrawal latency when it was co-injected with boiled IL-1β (Figure 7.3.).

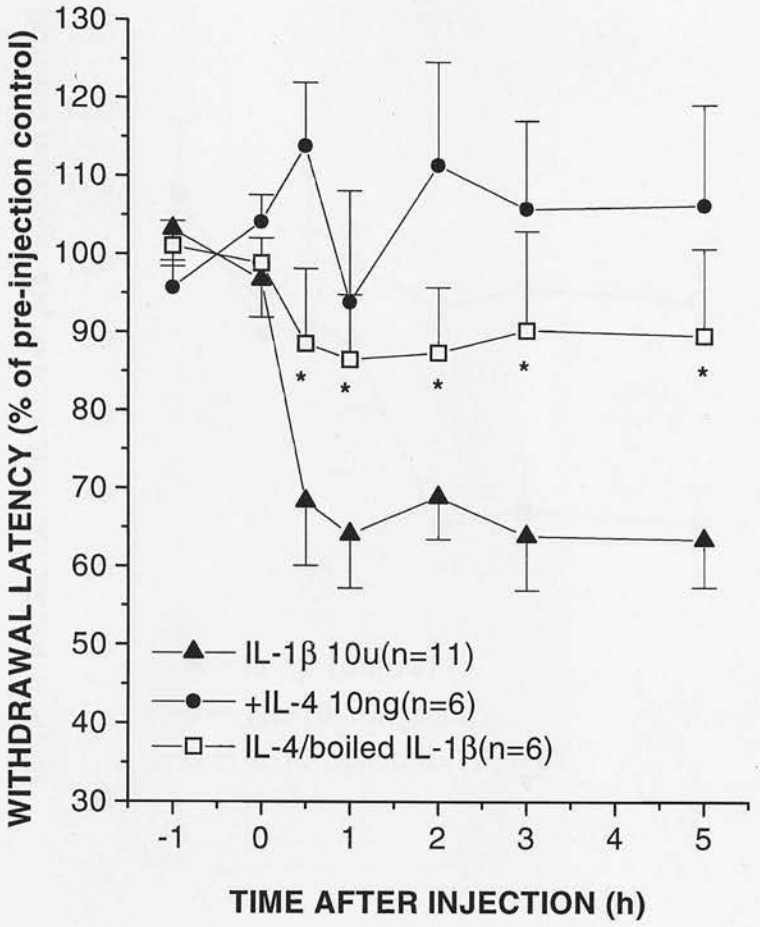


Figure 7.3

IL-4 reduces IL-1β–induced thermal hyperalgesia.

*Effects of i.pl. IL-1β alone, co-injected with IL-4 10ng and IL-4 10 ng co-injected with boiled IL-1β on withdrawal latency are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to IL-1 β alone.*

The reduction in withdrawal latency induced by i.pl. IL-1β (10 u) to a thermal stimulus was prevented by co-injection with IL-4 (10 ng), but only for 1h following injection. IL-10 had no significant effect on paw withdrawal latency when it was co-injected with boiled IL-1β (Figure 7.4.).

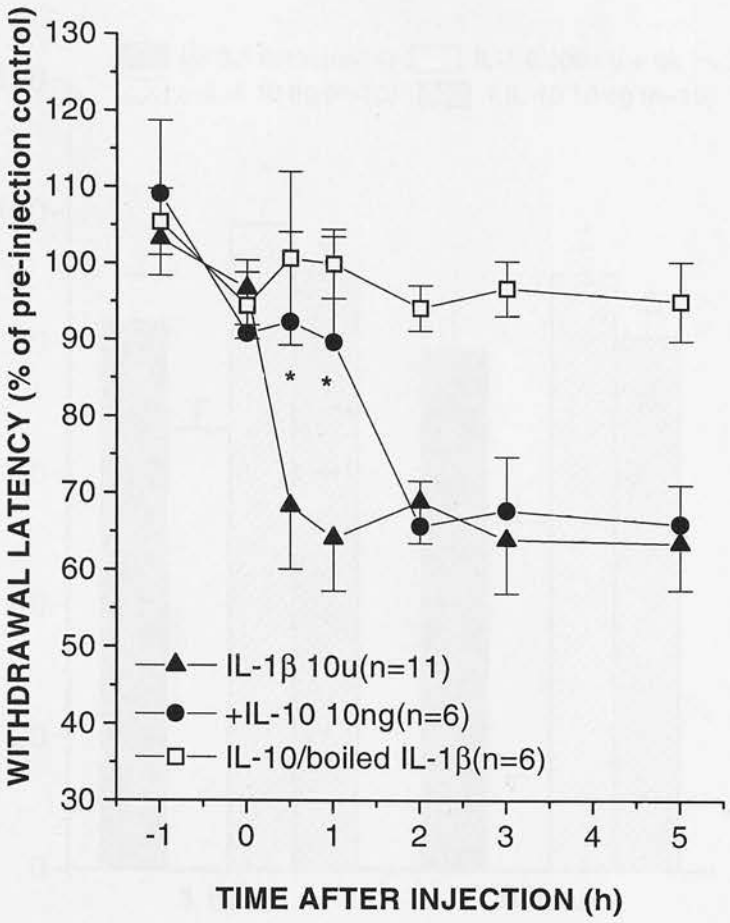


Figure 7.4

IL-10 only reduces IL-1β–induced thermal hyperalgesia for one hour.

*Effects of i.pl. IL-1β alone, co-injected with IL-10 10ng and IL-10 10 ng co-injected with boiled IL-1β on withdrawal latency are shown All values are mean ± s.e.m., * $p < 0.05$ compared to IL-1 β alone.*

Effects of IL-4 and IL-10 on IL-1β-induced effects on Bk-induced responsiveness in behavioural hyperalgesia.

The enhancement of Bk-induced (0.1nmol) hyperalgesia by IL-1β (0.001 u, i.art.) was prevented by both IL-4 10 ng and IL-10 10 ng for the duration of the experiement (Figure 7.5.).

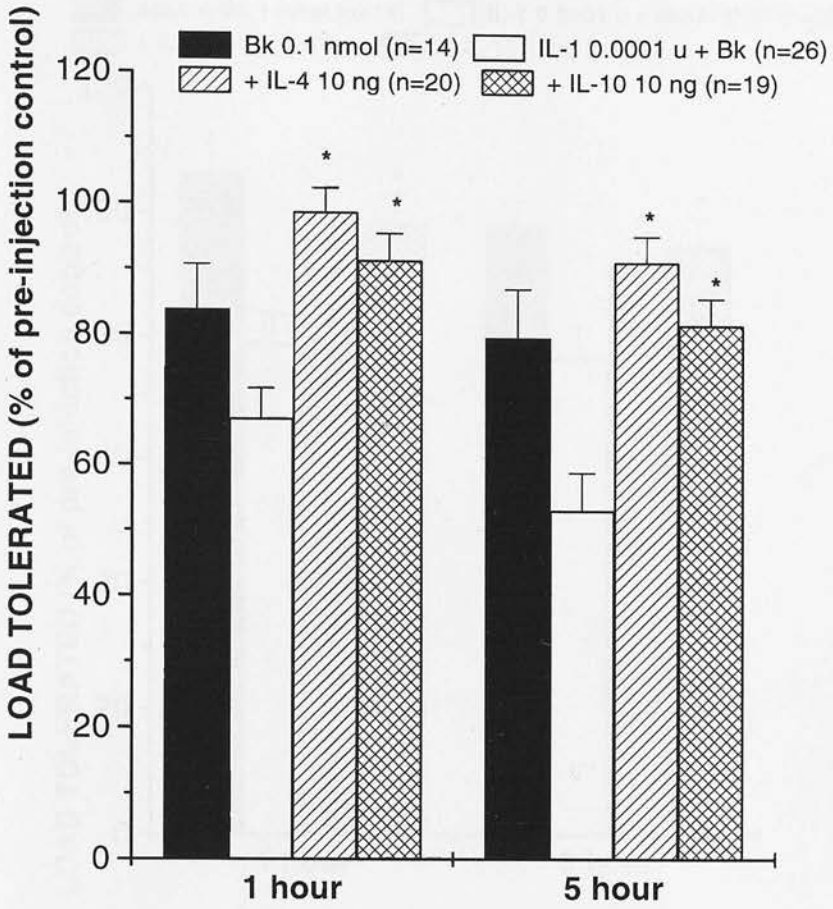


Figure 7.5

IL-4 and IL-10 block IL-1β–induced enhanced Bk-mediated mechanical hyperalgesia.

*Effects of IL-1β 0.001 u with Bk 0.1 nmol and co-injected with IL-4 10 ng or IL-10 10 ng on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to open bars*

Effects of IL-4 and IL-10 on IL-1β-induced effects on desArg^9-Bk-induced responsiveness in behavioural hyperalgesia.

Co-injection of IL-4 or IL-10 blocked the mechanical hyperalgesia induced co-injection of desArg^9-Bk 1 nmol and IL-1β 0.001 u, i.art throughout the course of the experiment (Figure 7.6).

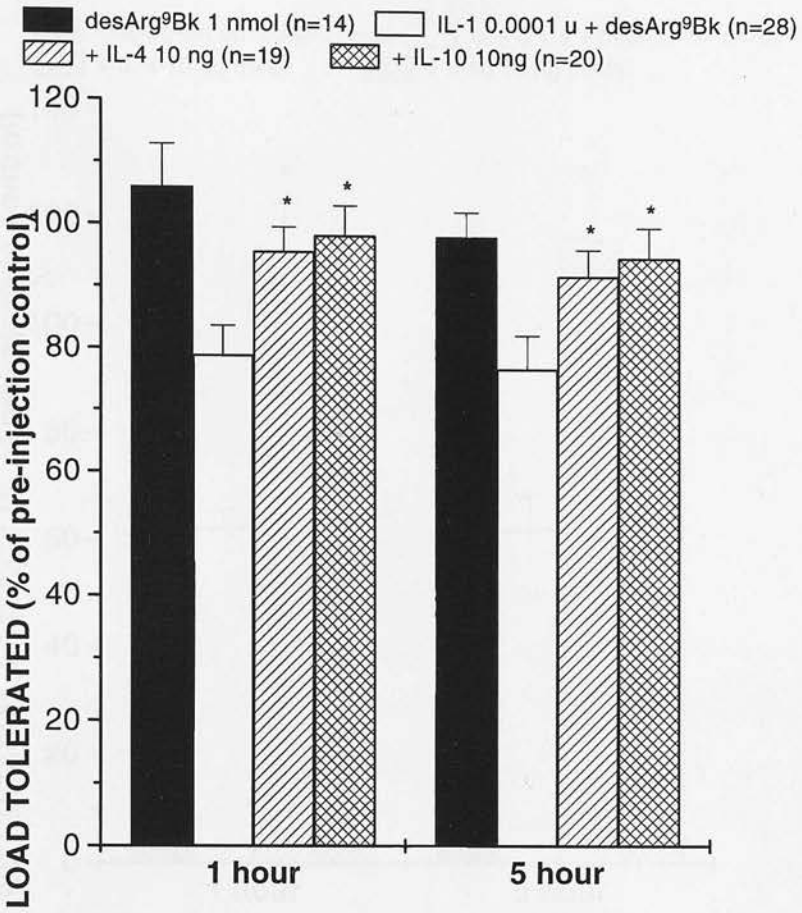


Figure 7.6

IL-4 and IL-10 block IL-1β–induced desArg^9-Bk-mediated mechanical hyperalgesia.

*Effects of IL-1β 0.001 u with desArg^9-Bk 1 nmol and co-injected with IL-4 10 ng or IL-10 10 ng on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to open bars*

The thermal hyperalgesia induced by co-injection desArg^9-Bk 50 nmol and IL-1β 0.1 u, i.pl. was prevented by IL-4 10 ng throughout the course of the experiment However, IL-10 10 ng only alleviated the hyperalgesia induced by co-injection of desArg^9-Bk 50 nmol and IL-1β 0.1 u, i.pl, 1 h after injection (Figure 7.7.).

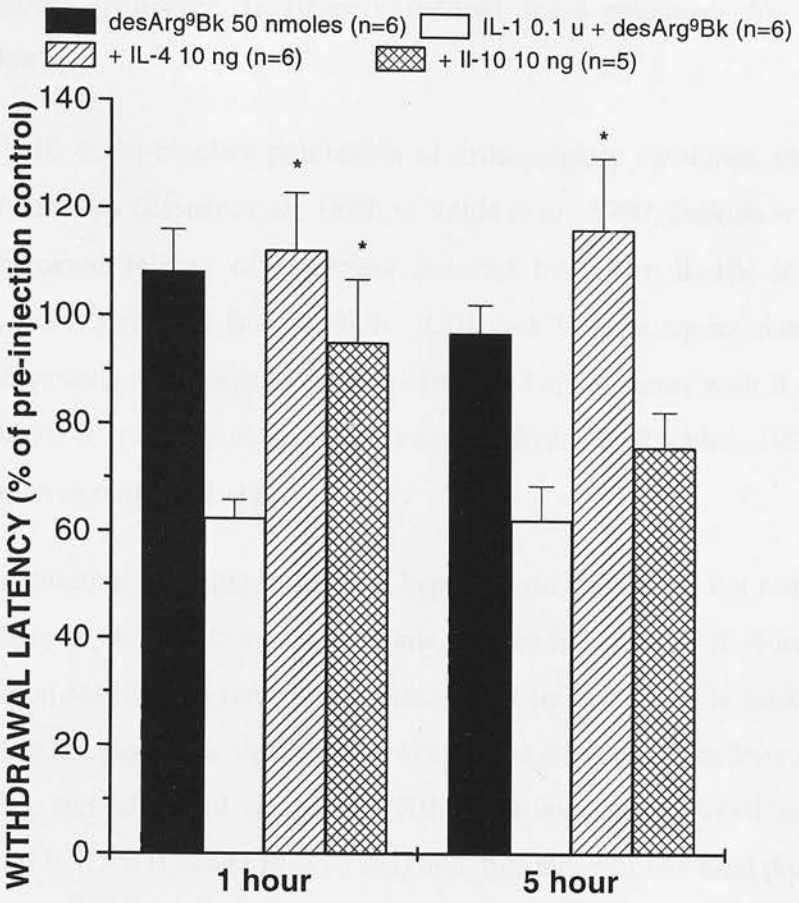


Figure 7.7

IL-4 and IL-10 block IL-1β–induced desArg^9-Bk-mediated thermal hyperalgesia with differing time courses.

*Effects of IL-1β 0.1 u with desArg^9-Bk 50 nmol and co-injected with IL-4 10 ng or IL-10 10 ng on load tolerated are shown. All values are mean ± s.e.m., * $p < 0.05$ compared to open bars*

7.4 Discussion

Both IL-4 and IL-10 reduced the hyperalgesia produced by local injection of IL-1β, as well as enhancement of responses to Bk and desArg^9-Bk induced by IL-1β in the mechanical hyperalgesia model. However differences in the time course of effect of IL-4 and IL-10 were observed in the thermal hyperalgesia model. IL-4 reduced both IL-1β-induced hyperalgesia as well as the induction of a kinin B_1 receptor-mediated hyperalgesia by IL-1β for up to 5h

following treatment. However, IL-10 only reduced these responses for the first hour following injection.

Both IL-4 and IL-10 down-regulate production of inflammatory cytokines, including IL-1β, from activated monocytes (Essner *et al.*, 1989; te Velde *et al.*, 1990; Jenkins *et al.*, 1994) thus reducing any increased release of cytokines induced by either IL-1β, itself, or kinins (Dinarello *et al.*, 1994; Tiffany & Burch, 1989). IL-10 and IL-4 also up-regulate synthesis and release of an endogenous antagonist to IL-1, IL-1ra. IL-1ra competes with IL-1β for the IL-1RI but once it binds it is unable to transduce a signal (Evans & Robbins, 1994), thus acting as an endogenous antagonist of IL-1β.

IL-4 has a longer duration of action in thermal hyperalgesia than IL-10 but not in mechanical hyperalgesia. There is no *in-vivo* evidence relating to the half- life of IL-4 and IL-10, but a possible explanation for the different time courses could be that IL-10 is broken down more rapidly than IL-4 in the paw. The differences between the two cytokines arise with respect to increased synthesis and release of soluble IL-1RII. IL-4 increases expression and release of the soluble form of IL-1RII (Colotta *et al.*, 1993) and this receptor can bind preferentially and irreversibly to IL-1β (Colotta *et al.*, 1994). Increased levels of this receptor would thus mop up the excess IL-1β and act as a decoy receptor reducing the biological activity of IL-1β. IL-4, but not IL-10 also regulated the production of inflammatory mediators, including PGE_2 from synoviocytes activated by IL-1β (Dechanet *et al.*, 1995). Thus, IL-4 has the ability to reduce the effects of IL-1β in a more diverse way than IL-10. Although this doesn't explain fully the difference in duration of action between IL-4 and IL-10, it does illustrate that they have different mechanisms by which they reduce the biological effects of IL-1β.

These results show that two anti-inflammatory cytokines can modulate the hyperalgesia induced by IL-1β and may play a role in modulating inflammatory hyperalgesia. These endogenously released mediators may play an autoregulatory role in the hyperalgesia and inflammation in clinical coditions such as rheumatoid arthritis.

8. DISCUSSION AND CONCLUSIONS

The future direction for the treatment of inflammatory joint diseases and the associated hyperalgesia appears to be focusing on the role of inflammatory mediators, which are increased in the synovial fluid of rheumatoid joints. In order to establish the exact role these mediators have in the associated hyperalgesia use will need to be made of experimental animal models. In this thesis I have used both electrophyisiological techniques (recording from afferent C-fibres) and behavioural hyperalgesia models to study the effects and interactions of two classes of inflammatory mediators, namely kinins and IL-1β.

These studies have shown that in inflammation, kinin B_1 receptors, which are not present (or if present, not functional) under normal physiological conditions, become important in the hyperalgesia associated with inflammation. From these present studies it is not possible to state that this is a direct effect via action on B_1 receptors located on sensory neurones, although it does seem improbable as studies have shown that there was no evidence of B_1 receptors on sensory neurones in cultured dorsal root ganglion cells following either injection Freund's adjuvant into neonatal rats (i.p.) or incubation of the cultured dorsal root ganglion cells with IL-1β (Davis *et al.*, 1996). It seems more likely that this B_1 mediated hyperalgesia is an indirect effect, mediated via B_1 receptors located on inflammatory cells. Such cells may include macrophages, fibroblasts and synovial cells (Tiffany & Burch, 1989; Lerner & Modeer, 1991; Bathon *et al.*, 1989). Activation of B_1 receptors on these cells would release inflammatory mediators which could act directly on nociceptors to alter their sensitivity and produce hyperalgesia.

Possible mediators released from inflammatory cells by kinin B_1 receptor agonists are the cytokines. IL-1β has been shown to activate sensory neurones (Fukuoka *et al.*, 1994) and can induce behavioural hyperalgesia (Davis & Perkins, 1994; Perkins & Kelly, 1994) which is consistent with the results presented in this thesis. This cytokine may therefore have a pivotal role in sensitising nociceptors. IL-1β is also involved in the induction of further B_1 receptors, an action that involves protein synthesis as it is blocked by cyclohexamide (De Blois *et al.*, 1991), which is consistent with results in this thesis. In these studies IL-1β also enhanced the responses induced by Bk. In behavioural studies the enhanced Bk-induced hyperalgesia is

blocked by both B_2 and B_1 receptor antagonists, suggesting that the enhanced hyperalgesia is mediated partly via B_1 receptors by the Bk breakdown product desArg^9-Bk. In neural experiments, this is less clear as the enhanced responses were completely blocked by a B_2 receptor antagonist. It would be interesting to investigate whether injection of a B_1 antagonist before IL-1β would prevent the enhancement of Bk-induced hyperalgesia.

IL-1β releases other cytokines including IL-1β itself. IL-1β also produces hyperalgesia via indirect mechanisms. For example, IL-1β can release prostanoids which have been shown to be involved in behavioural hyperalgesia (Cunha *et al.*, 1992) and PGE_2 and PGI_2 both activate knee joint C-fibres and sensitise them to Bk (Schepelmann *et al.*, 1992). IL-1 and B_1 agonists can act in synergy to increase levels of prostanoids. This is also consistent with the results in this thesis because indomethacin blocked IL-1β induced hyperalgesia, the enhanced hyperalgesia observed following Bk, and the B_1-mediated effects on neural discharge. However, in behavioural studies indomethacin did not block IL-1β-induced B_1-mediated hyperalgesia suggesting that other pathways can be involved. Another possibility are leukotrienes, in particular leukotriene B_4 (LTB4) - a product of the lipoxygenase pathway which has been shown to sensitise nociceptors to mechanical and thermal stimulus (Martin *et al.*, 1987). To study this, further experiments using inhibitors of 5-lipoxygenase, the enzyme responsible for generating leukotrienes from arachidonic acid, or specific leukotriene receptor antagonists. In recent years several 5-lipoxygense inhibitors and specific leukotriene antagonists have emerged. For example, zileuton (Abbot), a 5-lipoxygenase inhibitor (Rubin *et al.*, 1991) or ONO-4057 (ONO Pharmaceuticals) and CGS-25019C (Novartis), both LTB4 antagonists (for review see Girard and Bonne, 1997) could be emplyed to test this hypothesis.

With all the positive feedback loops occurring during inflammation (kinins releasing cytokines, cytokines enhancing responses to kinins and releasing further cytokines and both releasing prostanoids), it is not surprising that some negative feedback loops occur to autoregulate inflammation. The present studies have shown that endogenously released NO can inhibit neural discharge in nociceptors and can reduce Bk-induced activation of nociceptors in inflamed ankle joints. This is consistent with studies which have shown that NO is anti-nociceptive (Ferreira *et al*, 1991). Since kinins and IL-1β both induce the

production of NO, this may be one mechanism how inflammatory mediators reduce hyperalgesia associated with inflammation. The role of NO was not evaluated in arthritic knee joints, in which Bk responses had a tendency to be lower than those observed in normal knee joints. Thus it remains to be seen whether NO would have the same modulatory role in the knee joint or if this was an action peculiar to the inflamed ankle joint.

This thesis also highlights the negative feedback role of two cytokines, IL-4 and IL-10, which are both present in elevated levels in the synovial fluid obtained from patients with rheumatoid arthritis, and which can be released by IL-1β. In the present study IL-4 and IL-10 both prevented the hyperalgesia induced by IL-1β and IL-1β-induced kinin B_1 and B_2 receptor mediated hyperalgesia. This is consistent with previous reports that IL-10 inhibited cytokine-mediated hyperalgesia in the rat paw (Poole *et al.*, 1995). The exact mechanism by which IL-4 and IL-10 reduce hyperalgesia cannot be established from the experiments performed in this thesis. However, both have been show to inhibit IL-1 synthesis (Essner *et al.*, 1989), to increase levels of IL-1ra (Vannier *et al.*, 1992; Jenkins *et al.*, 1994) and increase levels of the soluble IL-1RII 'decoy receptor'(Colotta *et al.*, 1993), all of which would reduce the effects of IL-1β. Time constraints didn't permit evaluation of IL-4 and IL-10 in the electrophysiological experiments in this thesis, but it would obviously be of interest to study these in inflamed knee joints and could form the basis of exciting work in the future.

In conclusion although the results of experiments performed in this thesis go some way to establishing the role of certain inflammatory mediators in inflammatory pain, it seems unlikely that a single mediator is solely responsible for the associated hyperalgesia in inflammatory conditions such as rheumatoid arthritis. The inflammatory process is very complex with a cornucopia of mediators released which interact with each other and many more experiments are clearly required to further elucidate the underlying role of these mediators in the hyperalgesia associated with inflammatory pain. Hopefully results from such studies will lead to future therapies for inflammatory conditions such as rheumatoid arthritis. The main clinical treatment for conditions such as rheumatoid arthritis still remains the non-steroidal anti-inflammatory drugs (NSAIDS), these decrease the production of prostanoids by inhibiting their synthesis from arachidonic acid by inhibiting the COX enzyme. However, NSAIDS are not effective at alleviating the pain in all patients with rheumatoid arthritis and

often patients have periods of intense joint pain. There are other problems associated with inhibiting prostaglandin synthesis. Although NSAIDS may be effective at reducing the pain suffered by patients, they do not attenuate the on-going destruction of the joint, and so, leads to further irreversible damage to the joint. At the moment several pharmaceutical companies are researching new therapies to replace NSAIDS. The benefits of selective inhibitors of the inducible COX-2 enzyme, which have been shown to be anti-inflammatory without the typical side effects observed with COX-1 inhibitors, are being researched and developed for future use in the clinic (Talley, 1997), for example, celecoxib (Searle) (Hubbard *et al.*, 1996) and MK-966 (rofecoxib, Merck) (Ehrich *et al.*, 1996). Pharmaceutical companies are also actively considering the benefits of cytokine inhibitors including IL-1 receptor antagonists, interleukin converting enzyme inhibitors and anti-inflammatory cytokines for future clinical use in conditions such as rheumatoid arthritis, some of which are already undergoing clinical trials (for update see Earl, 1997). Orally active kinin receptor antagonists are also being researched and developed for future clinical use in conditions such as rheumatoid arthritis.

Future experiments.

As mentioned previously it is very difficult to ascertain the effects of antagonists on spontaneous (on-going) neural discharge in sensory nerves innervating inflamed knee joints. Further development of the rat knee joint model for recording from C-fibres is necessary, involving recording of mechanical- , or thermal-induced neural discharge in C-fibres which would be a closer correlate to the behavioural studies. Mechanical-induced effects on neural discharge have already been achieved in the cat knee joint (Schaible & Schmidt, 1983) where it was found that in inflamed joints normal physiological movement of the joint which would normally be non-noxious, induced activity in knee joint afferents. Although, the rat knee joint is much smaller than that in the cat, it seems possible that a movement-induced activaton of knee joint C-fibres could be achieved in this novel model. Indeed, pilot studies were carried out to see if this was possible and although technically more difficult, increases in neural discharge were observed following movement of the joint. However, further work should be carried out to enhance and improve the viability of such a model. If this was achieved the effects of antagonists could be studied, to test their behavioural anti-nociceptive actions in neural studies.

The main finding of this thesis has been that, in inflammation, kinin B_1 receptor agonists activate knee joint C-fibre nociceptors and induce a behavioural hyperalgesia. It seems likely that this is not via direct action via B_1 receptors located on nociceptors, but by an indirect action via B_1 receptors on inflammatory cells. It is not possible from the present results to establish the site of action of the B_1 receptor agonists. Kinin B_1 receptors have been shown to be present on macrophages, fibroblasts and synovial cells. Therefore, isolation and culture of the cells present within the knee joint following inflammation to investigate whether B_1 receptors are present on these cells should elucidate the site of action of B_1 receptor agonists in inflammatory hyperalgesia. It is possible to culture cells from synovial tissue and test whether these have B_1 kinin receptors by auto-radiography or functional studies.

The pain associated with inflammatory joint diseases, such as rheumatoid arthritis is literally crippling. Further studies like those suggested above are obviously worthwhile in attempting to improve our knowledge and understanding of the disorder and improve on existing treatments in the clinic.

9. REFERENCES.

AHLUWALIA, A. AND PERRETTI, M. (1996) Involvement of bradykinin b1 receptors in the polymorphonuclear leukocyte accumulation induced by il-1 beta in vivo in the mouse. *J. Immunol.* **156**, 269-274.

AKAHOSHI, T., OPPENHEIM, J.J., AND MATSUSHIMA, K. (1988) Interleukin 1 stimulates it's own receptor expression on human fibroblasts through the endogenous production of postaglandin(s). *J. Clin. Invest.* **82**, 1219-1224.

ARAI, K., NISHIDA, J., HAYASHIDA, K., HATAKE, K., KITAMURA, T., MIYAJIMA, A., ARAI, N., AND YOKOTA, T. (1990) [Coordinate regulation of immune and inflammatory responses by cytokines]. *Rinsho. Byori.* **38**, 347-353.

ASANO, M., INAMURA, N., HATORI, C., SAWAI, H., FUJIWARA, T., KATAYAMA, A., KAYAKIRI, H., SATOH, S., ABE, Y., INOUE, T., SAWADI, Y., NAKAHARI, K., OKU, T. AND OKUHARA, M. (1997) The identification of an orally active, non peptide bradykinin B_2 receptor antagonist, FR173657. *Br. J. Pharmacol.* **120**, 617-624.

BAREIS, D.L., MANGANIELLO, V.C., HIRATA, F., VAUGHAN, M., AND AXELROD, J. (1983) Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts. *Proc. Natl. Acad. Sci. USA* **80**, 2514-2518.

BATHON, J.M., MORELAND, L.W., AND DIBARTOLOMEO, A.G. (1989) Inflammatory central nervous system involvement in rheumatoid arthritis. *Semin. Arthritis Rheum.* **18**, 258-266.

BATHON, J.M., CROGHAN, J.C., MACGLASHAN, D.W., JR., AND PROUD, D. (1994) Bradykinin is a potent and relatively selective stimulus for cytosolic calcium elevation in human synovial cells. *J. Immunol.* **153**, 2600-2608.

BESSOU, P. AND PERL, E.R. (1969) Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *J. Neurophysiol.* **32**, 1025-1043.

BILLINGHAM, M.E. (1987) Cytokines as inflammatory mediators. *Br. Med. Bull.* **43**, 350-370.

BIRRELL, G.J., MCQUEEN, D.S., IGGO, A., AND GRUBB, B.D. (1990) The effects of 5-HT on articular sensory receptors in normal and arthritic rats. *Br. J. Pharmacol.* **101**, 715-721.

BIRRELL, G.J., MCQUEEN, D.S., IGGO, A., COLEMAN, R.A., AND GRUBB, B.D. (1991) PGI2-induced activation and sensitization of articular mechanonociceptors. *Neurosci. Lett.* **124**, 5-8.

BOLOTINA, V.M., NAJIBI, S., PALACINO, J.J., PAGANO, P.J. AND COHEN, R.J. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature.*, **368**, 850-853.

BOGLE, R.G., COADE, S.B., MONCADA, S., PEARSON, J.D. AND MANN, G.E. (1991). Bradykinin and ATP stimulate L-Arginine uptake and nitric oxide release in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **180**, 926-932.

BREDT, D.S., HWANG, P.M. AND SNYDER, S.H. (1990). Localisation of nitric oxide synthase indicating a neural role for nitric oxide. *Nature.*, **347**, 768-770.

BROWN, M., WEEB, M., PHILLIPS, E., SKIDMORE, E. AND MCINTYRE, P. (1995) Molecular studies on kinin receptors. *Can. J. Physiol. Pharmacol.* **73**, 780-786.

BOUTHILLIER, J., DEBLOIS, D., AND MARCEAU, F. (1987) Studies on the induction of pharmacological responses to des-Arg9- bradykinin in vitro and in vivo. *Br. J. Pharmacol.* **92**, 257-264.

BURCH, R.M. AND AXELROD, J. (1987) Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts: evidence for G protein regulation of phospholipase A2. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 6374-6378.

BURD, P.R., THOMPSON, W.C., MAX, E.E., AND MILLS, F.C. (1995) Activated mast cells produce interleukin 13. *J Exp Med* **181**, 1373-1380.

BURGER, D. AND DAYER, J.M. (1995) Inhibitory cytokines and cytokine inhibitors. *Neurology* **45**, Suppl 1994;6):S39-S43.

BURGESS, G.M., MULLANEY, I., MCNEILL, M., DUNN, P.M., AND RANG, H.P. (1989) Second messengers involved in the mechanism of action of bradykinin in sensory neurons in culture. *J. Neurosci.* **9**, 3314-3325.

CAHILL, M., FISHMAN, J.B. AND POLGAR, P. (1988). Effect of des Arginine^{9}-bradykinin and other bradykinin fragments on the synthesis of prostacyclin and the binding of bradykinin by vascular cells in culture. *Agents Actions,* **24**, 224-231.

CAMPBELL, J.N., RAJA, S.N., COHEN, R.H., MANNING, D.C., KHAN, A.A., AND MEYER, R.A. (1989) Peripheral neural mechanisms of nociception. In : *textbook of Pain,* 22-45. Edited by Wall AND Melzack, Churchll Livingstone, New York.

CAMPOS, M.M., SOUZA, G.E.P., AND CALIXTO, J.B. (1996) Upregulation of B-1 receptor mediating des-Arg(9)-Bk-induced rat paw oedema by systemic treatment with bacterial endotoxin Upregulation of B-1 receptor mediating des-Arg(9)-Bk-induced rat paw oedema by systemic treatment with bacterial endotoxin. *Br. J. Pharmacol.* **117**, 793-798.

CERRETTI, D.P., KOZLOSKY, C.J., MOSLEY, B., NELSON, N., VAN NESS, K., GREENSTREET, T.A., MARCH, C.J., KRONHEIM, S.R., DRUCK, T., CANNIZZARO, L.A., *ET AL* (1992) Molecular cloning of the interleukin-1 beta converting enzyme. *Science* **256**, 97-100.

CHEDID, M., SHIRAKAWA, F., NAYLOR, P., AND MIZEL, S.B. (1989) Signal transduction pathway for IL-1. Involvement of a pertussis toxin-sensitive GTP-binding protein in the activation of adenylate cyclase. *J. Immunol.* **142**, 4301-4306.

CHERCUITTE, F., BEAULIEU, A.D., POUBELLE, P., AND MARCEAU, F. (1987) Carboxypeptidase N (kininase I) activity in blood and synovial fluid from patients with arthritis. *Life Sci.* **41**, 1225-1232.

CODERRE, T.J. AND WALL, P.D. (1987) Ankle joint urate arthritis (AJUA) in rats: an alternative animal model of arthritis to that produced by Freund's adjuvant. *Pain* **28**, 379-393.

COLOTTA, F., RE, F., MUZIO, M., BERTINI, R., POLENTARUTTI, N., SIRONI, M., GIRI, J.G., DOWER, S.K., SIMS, J.E., AND MANTOVANI, A. (1993) Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* **261**, 472-475.

COLOTTA, F., DOWER, S.K., SIMS, J.E., AND MANTOVANI, A. (1994) The type ii 'decoy' receptor: a novel regulatory pathway for interleukin 1. *Immunol Today* **15**, 562-566.

COLOTTA, F., ORLANDO, S., FADLON, E.J., SOZZANI, S., MATTEUCCI, C., AND MANTOVANI, A. (1995) Chemoattractants induce rapid release of the interleukin 1 type ii decoy receptor in human polymorphonuclear cells. *J Exp Med* **181**, 2181-2188.

COLOTTA, F., SACCANI, S., GIRI, J.G., DOWER, S.K., SIMS, J.E., INTRONA, M., AND MANTOVANI, A. (1996) Regulated expression and release of the IL-1 decoy receptor in human mononuclear phagocytes Regulated expression and release of the IL-1 decoy receptor in human mononuclear phagocytes. *J. Immunol.* **156**, 2534-2541.

COMINELLI, F., NAST, C.C., CLARK, B.D., SCHINDLER, R., LIERENA, R., EYSSELEIN, V.E., THOMPSON, R.C., AND DINARELLO, C.A. (1990) Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J. Clin. Invest.* **86**, 972-980.

CORREA, C.R. AND CALIXTO, J.B. (1993) Evidence for participation of B1 and B2 kinin receptors in formalin- induced nociceptive response in the mouse. *Br. J. Pharmacol.* **110**, 193-198.

COSTELLO, A.H. AND HARGREAVES, K.M. (1989) Suppression of carrageenan-induced hyperalgesia, hyperthermia and edema by a bradykinin antagonist. *Eur. J. Pharmacol.* **171**, 259-263.

CUNHA, F.Q., POOLE, S., LORENZETTI, B.B., AND FERREIRA, S.H. (1992) The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br. J. Pharmacol.* **107**, 660-664.

D-ORLEANS-JUSTE, P., DE-NUCCI, G., AND VANE, J.R. (1989) Kinins act on B1 or B2 receptors to release conjointly endothelium- derived relaxing factor and prostacyclin from bovine aortic endothelial cells. *Br. J. Pharmacol.* **96**, 920-926.

DAVIS, A.J. AND PERKINS, M.N. (1994) The involvement of bradykinin B1 and B2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat. *Br. J. Pharmacol.* **113**, 63-68.

DAVIS, A.J. AND PERKINS, M.N. (1996) Substance P and capsaicin-induced mechanical hyperalgesia in the rat knee joint; The involvement of bradykinin B-1 and B-2 receptors. *Br. J. Pharmacol.* **118**, 2206-2212.

DE WAAL MALEFYT, R., ABRAMS, J., BENNETT, B., FIGDOR, C.G., AND DE VRIES, J.E. (1991) Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* **174**, 1209-1220.

DEBLOIS, D., BOUTHILLIER, J., AND MARCEAU, F. (1988) Effects of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to des-Argsup 9-bradykinin. *Br. J. Pharmacol.* **93**, 969-977.

DEBLOIS, D., BOUTHILLIER, J., AND MARCEAU, F. (1989) Pharmacological modulation of the up-regulated responses to desArg^9-bradykinin *in-vivo* and *in-vitro*. *Immunopharmacology*. **17**, 187-198.

DEBLOIS, D., BOUTHILLIER, J., AND MARCEAU, F. (1991) Pulse exposure to protein synthesis inhibitors enhances vascular responses to des-Arg9-bradykinin: Possible role of interleukin-1. *Br. J. Pharmacol.* **103**, 1057-1066.

DECHANET, J., RISSOAN, M.C., BANCHEREAU, J., AND MIOSSEC, P. (1995) Interleukin 4, but not interleukin 10, regulates the production of inflammation mediators by rheumatoid synoviocytes. *Cytokine* **7**, 176-183.

DINARELLO, C.A., SHPARBER, M., KENT, E.F., JR., AND WOLFF, S.M. (1981) Production of leukocytic pyrogen from phagocytes of neonates. *J. Infect. Dis.* **144**, 337-343.

DINARELLO, C.A. (1994) The interleukin-1 family: 10 years of discovery. *Faseb J* **8**, 1314-1325.

DRAY, A., BETTANEY, J., FORSTER, P., AND PERKINS, M.N. (1988) Activation of a bradykinin receptor in peripheral nerve and spinal cord in the neonatal rat in vitro. *Br. J. Pharmacol.* **95**, 1008-1010.

DRAY, A., PATEL, I.A., PERKINS, M.N., AND RUEFF, A. (1992) Bradykinin-induced activation of nociceptors: Receptor and mechanistic studies on the neontal rat spinal cord-tail preparation in vitro. *Br. J. Pharmacol.* **107**, 1129-1134.

DRAY, A. AND BEVAN, S. (1993) Inflammation and hyperalgesia: highlighting the team effort. *Trends. Pharmacol. Sci.* **14**, 287-290.

DUARTE, I.D.G., LORENZETTI, B.B. AND FERREIRA, S.H. (1990). Peripheral analgesia and activation of the nitric oxide cyclic GMP pathway. *Eur. J. Pharmacol.*, **186**, 289-293.

DUARTE, I.D.G., DOS SANTOS, I.R., LORENZETTI, B.B. AND FERREIRA, S.H. (1992). Analgesia by direct antagonism of nociceptor sensitization involves the Arginine-nitric oxide-cGMP pathway. *Eur. J. Pharmacol.*, **217**, 225-227.

DUNN, P.M. AND RANG, H.P. (1990) Bradykinin-induced depolarization of primary afferent nerve terminals in the neonatal rat spinal cord in vitro. *Br. J. Pharmacol.* **100**, 656-660.

EARL, J. (1997) Drug status update : A monthly update of the current status of drugs in development. *Rheumatoid Arthritis.* **1**, 493-502.

EHRICH, E., DALLOB, A., VAN HECKEN, A., DEPRE, M., DELEPELEIRE, I., BRIDEAU, C., HILLIARD, D., TANAKA, W., *ET AL.*. (1996) Demonstration of selective COX-2 inhibition by MK-966 in humans. *Arthritis Rheum.* **39 (Suppl 9)**, S81.

ESSNER, R., RHOADES, K., MCBRIDE, W.H., MORTON, D.L., AND ECONOMOU, J.S. (1989) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **142**, 3857-3861.

EVANS, S.W. AND WHICHER, J.T. (1992) An overview of the inflammatory response. In: *Biochemisty of Inflammation: Immunology and Medicine.* **18**, 1-15 Edited by K. Whaley. Kuwer Academic Publishers Dordtrecht/Boston/London

EVANS, C.H. AND ROBBINS, P.D. (1994) The interleukin-1 receptor antagonist and its delivery by gene transfer. *Receptor.* **4**, 9-15.

FARMER, S.G., BURCH, R.M., MEEKER, S.A., AND WILKINS, D.E. (1989) Evidence for a pulmonary B3 bradykinin receptor. *Mol. Pharmacol.* **36**, 1-8.

FARRELL, A.J., BLAKE, D.R., PALMER, R.M.J. AND MONCADA, S. (1992). Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann. Rheum. Dis.*, **51**, 1219-1222.

FENTON, M.J., VERMEULEN, M.W., CLARK, B.D., WEBB, A.C., AND AURON, P.E. (1988) Human pro-IL-1 beta gene expression in monocytic cells is regulated by two distinct pathways. *J. Immunol.,* **140**, 2267-2273.

FERREIRA, S.H., LORENZETTI, B.B., BRISTOW, A.F., AND POOLE, S. (1988) Interleukin-1beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. *Nature* **334**, 698-700.

FERREIRA, S.H. (1991) Cytokines and hyperalgesia. *Eur. J. Rheumatol. Inflam.* **11**, 133-138.

FERREIRA, S.H. AND VANE, J.R. (1967) The detection and estimation of bradykinin in the circulating blood. *Br. J. Pharmacol.* **29**, 367-377.

FIELDS, H.L. (1994) Peripheral neuropathic pain: an approach to management. In: *Handbook of Pain.* 991-996 Edited by P.D Wall and R. Melzack., Churchill Livingstone, London.

FIGUEROA, C.D., MACIVER, A.G., DIEPPE, P., MACKENZIE, J.C., AND BHOOLA, K.D. (1989) Presence of immunoreactive tissue kallikrein in human polymorphonuclear (PMN) leucocytes. *Adv. Exp. Med. Biol.* **247B**, 207-210.

FIGUEROA, C.D., HENDERSON, L.M., KAUFMANN, J., DE-LA-CADENA, R.A., COLMAN, R.W., MULLER-ESTERL, W., AND BHOOLA, K.D. (1992) Immunovisualization of high (HK) and low (LK) molecular weight kininogens on isolated human neutrophils. *Blood* **79**, 754-759.

FISCHER, E., MARANO, M.A., VAN ZEE, K.J., ROCK, C.S., HAWES, A.S., THOMPSON, W.A., DEFORGE, L., KENNEY, J.S., REMICK, D.G., BLOEDOW, D.C., *ET AL* (1992a) Interleukin-1 receptor blockade improves survival and hemodynamic performance in Escherichia coli septic shock, but fails to alter host responses to sublethal endotoxemia. *J. Clin. Invest.* **89**, 1551-1557.

FISCHER, E., VAN ZEE, K.J., MARANO, M.A., ROCK, C.S., KENNEY, J.S., POUTSIAKA, D.D., DINARELLO, C.A., LOWRY, S.F., AND MOLDAWER, L.L. (1992b) Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease. *Blood* **79**, 2196-2200.

FOLLENFANT, R.L., NAKAMURA-CRAIG, M., HENDERSON, B., AND HIGGS, G.A. (1989) Inhibition by neuropeptides of interleukin-1 beta-induced, prostaglandin-independent hyperalgesia. *Br. J. Pharmacol.* **98**, 41-43.

FUKUOKA, H., KAWATANI, M., HISAMITSU, T., AND TAKESHIGE, C. (1994) Cutaneous hyperalgesia induced by peripheral injection of interleukin-1 beta in the rat. *Brain Res.* **657**, 133-140.

GALIZZI, J.P., BODINIER, M.C., CHAPELAIN, B., LY, S.M., COUSSY, L., GIRAUD, S., NELIAT, G., AND JEAN, T. (1994) Up-regulation of (3H)-des-Arg10-kallidin binding to the bradykinin B1 receptor by interleukin-1beta in isolated smooth muscle cells: correlation with B1 agonist-ind. *Br. J. Pharmacol.* **113**, 389-394.

GIRARD, D., PAQUIN, R., NACCACHE, P.H., AND BEAULIEU, A.D. (1996) Effects of interleukin-13 on human neutrophil functions. *J. Leukoc. Biol.* **59**, 412-419.

GIRARD, J-P AND BONNE, C. (1997) Leukotriene B4 antagonists and the search for novel anti-inflammatory agents. *Curr. Res. Rheumatoid Arthritis.* **1**, 353-363.

GIROLAMI, J.P., ALHENC GELAS, F., DOS REIS, M.L., BASCANDS, J.L., SUC, J.M., CORVOL, P., AND MENARD, J. (1986) Hydrolysis of rat high molecular weight kininogen by purified rat urinary kallikrein: identification of bradykinin as the kinin formed. *Adv. Exp. Med. Biol.* **198 Pt A**, 137-145.

GRAVES, B.J., HATADA, M.H., HENDRICKSON, W.A., MILLER, J.K., MADISON, V.S., AND SATOW, Y. (1990) Structure of interleukin 1 alpha at 2.7-A resolution. *Biochemistry* **29**, 2679-2684.

GRIESBACHER, T., SUTLIFF, R.L., AND LEMBECK, F. (1994) Anti-inflammatory and analgesic activity of the bradykinin antagonist, icatibant (Hoe 140), against an extract from Porphyromonas gingivalis. *Br. J. Pharmacol.* **112**, 1004-1006.

GRIGG, P., SCHAIBLE, H.G., AND SCHMIDT, R.F. (1986) Mechanical sensitivity of group III and IV afferents from posterior articular nerve in normal and inflamed cat knee. *J. Neurophysiol.* **55**, 635-643.

GRONICH, J., KONIECZKOWSKI, M., GELB, M.H., NEMENOFF, R.A., AND SEDOR, J.R. (1994) Interleukin 1 alpha causes rapid activation of cytosolic phospholipase A2 by phosphorylation in rat mesangial cells. *J. Clin. Invest.* **93**, 1224-1233.

GRUBB, B.D., MCQUEEN, D.S., IGGO, A., BIRRELL, G.J., AND DUTIA, M.B. (1988) A study of 5-HT-receptors associated with afferent nerves located in normal and inflamed rat ankle joints. *Agents Actions* **25**, 216-218.

GUILBAUD, G., IGGO, A., AND TEGNER, R. (1985) Sensory receptors in ankle joint capsules of normal and arthritic rats. *Exp. Brain Res* **58**, 29-40.

GUTMAN, N., MOREAU, T., ALHENC-GELAS, F., BAUSSANT, T., ELMOUJAHED, A., AKPONA, S., and GAUTHIER, F. (1988) T-kinin release from T-kininogen by rat submaxillary gland endopeptidase K. *Eur. J. Pharmacol.* **171**, 577-582.

GUY, G.R., CHUA, S.P., WONG, N.S., NG, S.B., AND TAN, Y.H. (1991) Interleukin 1 and tumor necrosis factor activate common multiple protein kinases in human fibroblasts. *J. Biol. Chem.* **266**, 14343-14352.

HALEY, J.E., DICKENSON, A.H., AND SCHACHTER, M. (1989) Electrophysiological evidence for a role of bradykinin in chemical nociception in the rat. *Neurosci. Lett.* **97**, 198-202.

HALEY, J.E., DICKENSON, A.H. AND SCHACHTER, M. (1992) Electrophysiological evidence for a role of nitric oxide in prologed chemical nociception in the rat. *Neuropharmacol.*, **31**, 251-258.

HARDY, J.D., WOLFF, H.G., AND GOODELL, H. (1952) In : *Pain sensation and reactions*. Wiliams and Wilkins, Baltimore, Maryland.

HARGREAVES, K.M., TROULLOS, E.S., DIONNE, R.A., SCHMIDT, E.A., SCHAFER, S.C., AND JORIS, J.L. (1988) Bradykinin is increased during acute and chronic inflammation: therapeutic implications. *Clin. Pharmacol. Ther.* **44**, 613-621.

HEPPELMANN, B., PFEFFER, A., SCHAIBLE, H.G., AND SCHMIDT, R.F. (1986) Effects of acetylsalicylic acid and indomethacin on single groups III and IV sensory units from acutely inflamed joints. *Pain* **26**, 337-351.

HERBERT, M.K. AND SCHMIDT, R.F. (1992) Activation of normal and inflamed fine articular afferent units by serotonin. *Pain* **50**, 79-88.

HESS, J.F., BORKOWSKI, J.A., YOUNG, G.S., STRADER, C.D., AND RANSOM, R.W. (1992) Cloning and pharmacological characterization of a human bradykinin (Bk-2) receptor. *Biochem. Biophys. Res. Commun.* **184**, 260-268.

HILDEBRAND, C., OQVIST, G., BRAX, L., AND TUISKU, F. (1991) Anatomy of the rat knee joint and fibre composition of a major articular nerve. *Anat. Rec.* **229**, 545-555.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE-ST, BREIPOHL, G., KONIG, W., KNOLLE, J., AND SCHOLKENS, B.A. (1991) Hoe 140 a new potent and long acting bradykinin-antagonist: In vitro studies. *Br. J. Pharmacol.* **102**, 769-773.

HUBBARD, R.C., MEHLISCH, D.R., JASPER, D.R., NUGENT, N.J., YU, S. AND ISAKSON, P.C. (1996) SC-58635, a highly selective inhibitor of COX-2, is an effective analgesic in an acute post-surgical pain model. *J. Invest. Med.* **44 (3)**, 293A.

HUGHES, S.R., WILLIAMS, T.J., AND BRAIN, S.D. (1990) Evidence that endogenous nitric oxide modulates oedema formation induced by substance P. *Eur. J. Pharmacol.* **191**, 481-484.

JANSEN, K., TUXEN, C., PEDERSEN-BJERGAARD, U., JANSEN, I., EDVINSSON, L., AND OLESEN, J. (1990) Pain, wheal and flare in human forearm skin induced by bradykinin and 5-hydroxytryptamine. *Peptides* **11**, 1133-1138.

JASANI, M.K., KATORI, M. AND LEWIS, G.P. (1969). Intracellular enzymes and kinin enzymes in synovial fluid in joint diseases. *Am. Rheum. Dis.*, **28**, 497-511.

JENKINS, J.K., MALYAK, M., AND AREND, W.P. (1994) The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. *Lymphokine Cytokine. Res.* **13**, 47-54.

JOYCE, D.A., GIBBONS, D.P., GREEN, P., STEER, J.H., FELDMANN, M., AND BRENNAN, F.M. (1994) Two inhibitors of pro-inflammatory cytokine release, interleukin-10 and interleukin-4, have contrasting effects on release of soluble p75 tumor necrosis factor receptor by cultured monocytes. *Eur. J. Immunol.* **24**, 2699-2705.

KANAKA, R., SCHAIBLE, H.G., AND SCHMIDT, R.F. (1985) Activation of fine articular afferent units by bradykinin. *Brain Res* **327**, 81-90.

KAWABATA, A., UMEDA, N. AND TAKAGI, H. (1993). L-Arginine plays adual role in nociceptive processing in the brain: involvement of the kyrotrophin-met-enkephalin pathway and NO-cyclicGMP pathway. *Br. J. Pharmacol.*, **109**, 73-79.

KAWABATA, A., MANABE, S., MANABE, Y. AND TAKAGI, H. (1994). Effect of topical administration of l-Arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. *Br. J. Pharmacol.*, **112**, 547-550.

KAWATANI, M. AND BIRDER, L. (1992) Interleukin-1 facilitates Ca2+ release in acutely dissociated dorsal root ganglion (DRG) cells of rat. *Neurosci. Abstr.* **18**, 691(Abstract)

KELLY, D.C., ASGHAR, A.U.R., MARR, C.G., McQUEEN, D.S. AND PERKINS, M.N. (1994). Actions of nitric oxide and endothelin on the activity of articular nociceptors in normal and inflamed ankle joints in the anaesthetised rat. *Br. J. Pharmacol.*, **113**, 72P

KESTER, M., SIMONSON, M.S., MENE, P., AND SEDOR, J.R. (1989) Interleukin-1 generates transmembrane signals from phospholipids through novel pathways in cultured rat mesangial cells. *J. Clin. Invest.* **83**, 718-723.

KHASAR, S.G., GREEN, P.G., AND LEVINE, J.D. (1993) Comparison of intradermal and subcutaneous hyperalgesic effects of inflammatory mediators in the rat. *Neurosci. Lett.* **153**, 215-218.

KINDGENMILLES, D. (1995) Effects of prostaglandin E(2) on the intensity of bradykinin-evoked pa in from skin and veins of humans. *Eur. J. Pharmacol.* **294**, 491-496.

KOCH, A.E., KUNKEL, S.L., CHENSUE, S.W., HAINES, G.K., AND STRIETER, R.M. (1992) Expression of interleukin-1 and interleukin-1 receptor antagonist by human rheumatoid synovial tissue macrophages. *Clin. Immunol. Immunopathol.* **65**, 23-29.

KUMAZAWA, T., MIZUMURA, K., AND KODA, H. (1993) Involvement of EP3 subtype of prostaglandin E receptors in PGE2-induced enhancement of the bradykinin response of nociceptors. *Brain Res.* **632**, 321-324.

KUMAZAWA, T., MIZUMURA, K., AND KODA, H. (1994) Possible involvement of the EP2 receptor subtype in PGE2-induced enhancement of the heat response of nociceptors. *Neurosci. Lett.* **175**, 71-73.

KURT JONES, E.A., BELLER, D.I., MIZEL, S.B., AND UNANUE, E.R. (1985) Identification of a membrane associated interleukin 1 in macrophages. *Proc. Nat. Acad. Sci. USA.* **82**, 1204-1208.

LANGFORD, L.A. AND SCHMIDT, R.F. (1983). Afferent and efferent axons in the medial and posterior articular nerves of the cat. *Anat. Rec.*, **206**, 71-78.

LERNER, U.H. AND MODEER, T. (1991) Bradykinin B1 and B2 receptor agonists synergistically potentiate interleukin-1-induced prostaglandin biosynthesis in human gingival fibroblasts. *Inflammation* **15**, 427-436.

LEVESQUE, L., DRAPEAU, G., GROSE, J.H., RIOUX, F., AND MARCEAU, F. (1993) Vascular mode of action of kinin B1 receptors and development of a cellular model for the investigation of these receptors. *Br. J. Pharmacol.* **109**, 1254-1262.

LEWIS, G.P. AND WINSEY, N.J. (1970) The action of pharmacologically active substances on the flow and composition of cat hind limb lymph. *Br. J. Pharmacol.* **40**, 446-460.

LI, P., ALLEN, H., BANERJEE, S., FRANKLIN, S., HERZOG, L., JOHNSTON, C., MCDOWELL, J., PASKIND, M., RODMAN, L., SALFELD, J., TOWNE, E., TRACEY, D., WARDWELL, S., WEI, F.Y., WONG, W., KAMEN, R., AND SESHADRI, T. (1995) Mice deficient in il-1 beta-converting enzyme are defective in production of mature il-1 beta and resistant to endotoxic shock. *Cell* **80**, 401-411.

MACNEIL, I.A., SUDA, T., MOORE, K.W., MOSMANN, T.R., AND ZLOTNIK, A. (1990) IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol.* **145**, 4167-4173.

MANDLE, R.J., COLMAN, R.W., AND KAPLAN, A.P. (1976) Identification of prekallikrein and high-molecular-weight kininogen as a complex in human plasma. *Proc. Natl. Acad. Sci. U. S. A.* **73**, 4179-4183.

MANNING, D.C., RAJA, S.N., MEYER, R.A., AND CAMPBELL, J.N. (1991) Pain and hyperalgesia after intradermal injection of bradykinin in humans. *Clin. Pharmacol. Ther.* **50**, 721-729.

MARCEAU, F., LUSSIER, A., REGOLI, D., AND GIROUD, J.P. (1983) Pharmacology of kinins: their relevance to tissue injury and inflammation. *Gen. Pharmacol.* **14**, 209-229.

MARCEAU, F. (1995) Kinin b-1 receptors: a review. *Immunopharmacology* **30**, 1-26.

MARINOZZI, G., FERRANTE, F., GAUDIO, E., RICCI, A., AND AMENTA, F. (1991) Intrinsic innervation of the rat knee joint articular capsule and ligaments. *Acta Anat. Basel.* **141**, 8-14.

MARSH, K.A. AND HILL, S.J. (1994) Des-Arg9-bradykinin-induced increases in intracellular calcium ion concentration in single bovine tracheal smooth muscle cells. *Br. J. Pharmacol.* **112**, 934-938.

MARTIN, H.A., BASBAUM, A.I., KWIAT, G.C., GOETZL, E.J., AND LEVINE, J.D. (1987) Leukotriene and prostaglandin sensitization of cutaneous high-threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. *Neuroscience* **22**, 651-659.

MATHIAS, S., YOUNES, A., KAN, C.C., ORLOW, I., JOSEPH, C., AND KOLESNICK, R.N. (1993) Activation of sphingomyelin signalling pathway in intact EL4 cells and in a cell free system by IL-1β. *Science* **259**, 519-522.

MCEACHERN, A.E., SHELTON, E.R., BHAKTA, S., OBERNOLTE, R., BACH, C., ZUPPAN, P., FUJISAKI, J., ALDRICH, R.W., AND JARNAGIN, K. (1991) Expression cloning of a rat B2 bradykinin receptor. *Proc. Natl. Acad. Sci. U. S. A.* **88**, 7724-7728.

MCGEHEE, D.S., GOY, M.F., AND OXFORD, G.S. (1992) Involvement of the nitric oxide-cyclic GMP pathway in the desensitization of bradykinin responses of cultured rat sensory neurons. *Neuron* **9**, 315-324.

MCGUIRK, S.M. AND DOLPHIN, A.C. (1992) G-protein mediation in nociceptive signal transduction: An investigation into the excitatory action of bradykinin in a subpopulation of cultured rat s. *Neuroscience* **49**, 117-128.

MCINTYRE, K.W., STEPAN, G.J., KOLINSKY, K.D., BENJAMIN, W.R., PLOCINSKI, J.M., KAFFKA, K.L., CAMPEN, C.A., CHIZZONITE, R.A., AND KILIAN, P.L. (1991) Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. *J. Exp. Med.* **173**, 931-939.

MCMAHAN, C.J., SLACK, J.L., MOSLEY, B., COSMAN, D., LUPTON, S.D., BRUNTON, L.L., GRUBIN, C.E., WIGNALL, J.M., JENKINS, N.A., BRANNAN, C.I., *ET AL* (1991) A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *EMBO J.* **10**, 2821-2832.

MCMAHON, S. AND KOLTZENBURG, M. (1990) The changing role of primary afferent neurones in pain [editorial]. *Pain* **43**, 269-272.

MENKE, J.G., BORKOWSKI, J.A., BIERILO, K.K., MACNEIL, T., DERRICK, A.W., SCHNECK, K.A., RANSOM, R.W., STRADER, C.D., LINEMEYER, D.L., AND HES (1994) Expression cloning of a human B1 bradykinin receptor. *J. Biol. Chem.* **269**, 21583-21586.

MENSE, S. AND MEYER, H. (1985) Different types of slowly conducting afferent units in cat skeletal muscle and tendon. *J. Physiol. (Lond)* **363**, 403-417.

MESSLINGER, K., SCHEPELMANN, K., PAWLAK, M., AND SCHMIDT, R.F. (1993) Bradykinin B1 and B2 receptor antagonists do not change the ongoing activity of slowly conducting articular afferents in the inflamed knee joint of the cat. *Neurosci. Lett.* **164**, 21-24.

MINTY, A., CHALON, P., DEROCQ, J.M., DUMONT, X., GUILLEMOT, J.C., KAGHAD, M., LABIT, C., LEPLATOIS, P., LIAUZUN, P., MILOUX, B., *ET AL* (1993) Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* **362**, 248-250.

MIZEL, S.B. (1990) How does interleukin 1 activate cells? Cyclic AMP and interleukin 1 signal transduction [see comments]. *Immunol. Today* **11**, 390-391.

MIZUMURA, K., MINAGAWA, M., TSUJII, Y., AND KUMAZAWA, T. (1990) The effects of bradykinin agonists and antagonists on visceral polymodal receptor activities. *Pain* **40**, 221-227.

MONCADA, S., PALMER, R.M.J. AND HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43(2)**, 109-142.

MULLER ESTERL, W., LOTTSPEICH, F., KELLERMANN, J., AND HENSCHEN, A. (1986) On the evolution of mammalian kininogens. *Behring. Inst. Mitt.* 48-61.

MUNOZ, E., BEUTNER, U., ZUBIAGA, A., AND HUBER, B.T. (1990) IL-1 activates two separate signal transduction pathways in T helper type II cells. *J. Immunol.* **144**, 964-969.

NEWBOULD, B.B. (1963) Chemotherapy of arthritis induced in rats byinjection of mycobacterial adjuvant. *Br. J. Pharmacol.* **21**, 127-136.

NUSTAD, K., VAAJE, K., AND PIERCE, J.V. (1975) Synthesis of kallikreins by rat kidney slices. *Br. J. Pharmacol.* **53**, 229-234.

O'NEILL, L.A. AND LEWIS, G.P. (1989) Interleukin-1 potentiates bradykinin- and TNF alpha-induced PGE2 release. *Eur. J. Pharmacol.* **166**, 131-137.

O'NEILL, L.A.J. (1995) Towards an understanding of the signal transduction pathways for interleukin 1. *Bba-Mol Cell Res* **1266**, 31-44.

O'NEILL, L.A.J. (1997) Molecular mechanisms underlying the actions of the pro-inflammatory cytokine interleukin i. *Biochemical Society Transactions* **25**, 295-302.

OGINO, K., HATANAKA, K., KAWAMURA, M., KATORI, M. AND HARADA, Y (1997) Evaluation of the pharmacological profile of meloxicam as an anti-inflammatory agent, with particular reference to its relative selectivity for cyclooxygenase-2 over cyclooxygenase-1. *Pharmacology*. **55**, 44-53.

OKAMOTO, H. AND GREENBAUM, L.M. (1983) Isolation and structure of T-kinin. *Biochem. Biophys. Res. Commun.* **112**, 701-708.

PERKINS, M.N., CAMPBELL, E., AND DRAY, A. (1993) Antinociceptive activity of the bradykinin B1 and B2 receptor antagonists, desArg9Leu8Bk and Hoe 140, in two models of persistent hypealgesia in the rat. *Pain* 191-197.

PERKINS, M.N. AND KELLY, D. (1993) Induction of bradykinin B1 receptors in vivo in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br. J. Pharmacol.* **110**, 1441-1444.

PERKINS, M.N. AND KELLY, D. (1994) Interleukin-1 beta induced-desArg9bradykinin-mediated thermal hyperalgesia in the rat. *Neuropharmacology* **33**, 657-660.

PFEILSCHIFTER, J., ROB, P., MULSCH, A., FANDRY, J., VOSBECK, K. AND BUSSE, R. (1992). Interleukin 1β and tumour necrosis factor a induce a macrophage-type of nitric oxide synthase in rat renal mesangial cells. *Eur. J. Pharmacol.*, **203**, 251-255.

POOLE, S., CUNHA, F.Q., SELKIRK, S., LORENZETTI, B.B., AND FERREIRA, S.H. (1995) Cytokine-mediated inflammatory hyperalgesia limited by interleukin-10. *Br. J. Pharmacol.* **115**, 684-688.

PRIESTLE, J.P., SCHAR, H.P., AND GRUTTER, M.G. (1989) Crystallographic refinement of interleukin 1 beta at 2.0 A resolution. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 9667-9671.

PROUD, D., BAUMGARTEN, C.R., NACLERIO, R.M., AND WARD, P.E. (1987) Kinin metabolism in human nasal secretions during experimentally induced allergic rhinitis. *J. Immunol.* **138**, 428-434.

RAJA, S.N., MEYER, R.A., AND CAMPBELL, J.N. (1988) Peripheral mechanisms of somatic pain. *Anesthesiology.* **68**, 571-590).

RANDALL, L.O. AND SELITTO, J.J. (1957) A method for measurement of analgesic activity in inflamed tissue. *Arch. Int. Pharmacodyn.* **111**, 409-419.

RANG, H.P., BEVAN, S., AND DRAY, A. (1991) Chemical activation of nociceptive peripheral neurones. *Br. Med. Bull.* **47**, 534-548.

RE, F., MUZIO, M., DE ROSSI, M., POLENTARUTTI, N., GIRI, J.G., MANTOVANI, A., AND COLOTTA, F. (1994) The type II "receptor" as a decoy target for interleukin-1 in polymorphonuclear leukocytes: Characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J. Exp. Med.* **179**, 739-743.

REGOLI, D., BARABE, J., AND PARK, W.K. (1977a) Receptors for bradykinin in rabbit aortae. *Can J Physiol Pharmacol.* **55**, 855-867.

REGOLI, D., RIZZI, A., CALO, G., NSA ALOGHA, S., AND GOBEIL, F. (1977b) B1 and B2 kinin receptors in various species. *Immunopharmacol.* **36**, 143-147.

REGOLI, D., RHALEB, N.E., DRAPEAU, G., DION, S., TOUSIGNANT, C., D'ORLEANS JUSTE, P., AND DEVILLIER, P. (1989) Basic pharmacology of kinins: pharmacologic receptors and other mechanisms. *Adv. Exp. Med. Biol.* **247A**, 399-407.

REGOLI, D., JUKIC, D., TOUSIGNANT, C., AND RHALEB, N.E. (1992) Kinin receptor classification. *Agents Actions* **38**, 475-486.

REGOLI, D., JUKIC, D., RHALEB, N.E. AND GOBEIL, F. (1992) Receptor for bradykinin and related kinins. A critical analysis. *Can. J. Pharmacol. Physiol.* **71**, 556-567.

REHBOCK, J., STEINHAUSER, C., AND HERMANN, A. (1992) Quantification of Ile-Ser-Bradykinin degradation in human serum and ascites. *Agents Actions* **38**, 449-453.

ROBERTS, R.A. AND GULLICK, W.J. (1990) Bradykinin receptors undergo ligand-induced desensitization. *Biochemistry* **29**, 1975-1979.

ROSOFF, P.M., SAVAGE, N., AND DINARELLO, C.A. (1988) Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism. *Cell* **54**, 73-81.

ROTHWELL, N. AND HOPKINS, (1995) Cytokines and the nervous system II: Actions and mechanisms of action. *TINS,* **18**, 130-136.

RUBIN, P., DUBE, L., BRAEKMAN, R., SWANSON, L., HANSEN, R., ALBERT, D. AND CARTER, G. (1991) Pharmacokinetics, safety, and ability to diminish leukotriene synthesis by zileuton, an inhibitor of 5-lipoxygenase. *Agents Actions* **35 Suppl,** 103 -116

SAKAMOTO, W., SATOH, F., NAGASAWA, S., AND HANDA, H. (1988) Identification of T-kinin-Leu(T-kinin-containing peptide) released from T-kininogen by cathepsin D of granulomatous tissues in rats. *Biochem. Biophys. Res Commun.* **150**, 1199-1206.

SATO, E., KOYAMA, S., NOMURA, H., KUBO, K., AND SEKIGUCHI, M. (1996) Bradykinin stimulates alveolar macrophages to release neutrophil, monocyte, and eosinophil chemotactic activity. *J. Immunol.* **157**, 3122-3129.

SAWUTZ, D.G., SALVINO, J.M., DOLLE, R.E., CASIANO, F., WARD, S.J., HOUCK, W.T., FAUNCE, D.M., DOUTY, B.D., BAIZMAN, E., AND AWAD, M.M. (1994) The nonpeptide WIN 64338 is a bradykinin B2 receptor antagonist. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4693-4697.

SCHAIBLE, H.G. AND GRUBB, B.D. (1993) Afferent and spinal mechanisms of joint pain. *Pain* **55**, 5-54.

SCHAIBLE, H.G. AND SCHMIDT, R.F. (1983) Activation of groups III and IV sensory units in medial articular nerve by local mechanical stimulation of knee joint. *J. Neurophysiol.* **49**, 35-44.

SCHAIBLE, H.G. AND SCHMIDT, R.F. (1985) Effects of an experimental arthritis on the sensory properties of fine articular afferent units. *J. Neurophysiol.* **54**, 1109-1122.

SCHAIBLE, H.G. AND SCHMIDT, R.F. (1988) Excitation and sensitization of fine articular afferents from cat's knee joint by prostaglandin E2. *J. Physiol.* **403**, 91-104.

SCHEPELMANN, K., MESSLINGER, K., SCHAIBLE, H.G., AND SCHMIDT, R.F. (1992) Inflammatory mediators and nociception in the joint: excitation and sensitization of slowly conducting afferent fibers of cat's knee by prostaglandin I2. *Neuroscience* **50**, 237-247.

SCHWAB, J.H., ANDERLE, S.K., BROWN, R.R., DALLDORF, F.G., AND THOMPSON, R.C. (1991) Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. *Infect. Immun.* **59**, 4436-4442.

SECKINGER, P., WILLIAMSON, K., BALAVOINE, J.F., MACH, B., MAZZEI, G., SHAW, A., AND DAYER, J.M. (1987) A urine inhibitor of interleukin 1 activity affects both interleukin 1 alpha and 1 beta but not tumor necrosis factor alpha. *J. Immunol.* **139**, 1541-1545.

SECKINGER, P., YARON, I., MEYER, F.A., YARON, M., AND DAYER, J.M. (1990) Modulation of the effects of interleukin-1 on glycosaminoglycan synthesis by the urine-derived interleukin-1 inhibitor, but not by interleukin-6. *Arthritis Rheum.* **33**, 1807-1814.

SHARMA, J.N., ZEITLIN, I.J., DEODHAR, S.D., AND BUCHANAN, W.W. (1983) Detection of kallikrein-like activity in inflamed synovial tissue. *Arch. Int. Pharmacodyn. Ther.* **262**, 279-286.

SHARMA, J.N. (1993). Inhibition of adjuvant inflammation in rats by the kinin receptor antagonist Hoe 140. 5th Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators (INWIN), Geneva (Switzerland).

SHERRINGTON, C.S. (1906). The interactive actions of the central nervous system. London: Constable.

SIMS, J.E., MARCH, C.J., COSMAN, D., WIDMER, M.B., MACDONALD, H.R., MCMAHAN, C.J., GRUBIN, C.E., WIGNALL, J.M., JACKSON, J.L., CALL, S.M., *ET AL* (1988) cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* **241**, 585-589.

SIMS, J.E., GAYLE, M.A., SLACK, J.L., ALDERSON, M.R., BIRD, T.A., GIRI, J.G., COLOTTA, F., RE, F., MANTOVANI, A., AND SHANEBECK, K. (1993) Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6155-6159.

SIMS, J.E., PAINTER, S.L., AND GOW, I.R. (1995) Genomic organization of the type I and type II IL-1 receptors. *Cytokine.* **7**, 483-490.

SMERZ-BERTLING, C. AND DUSCHL, A. (1995) Both interleukin 4 and interleukin 13 induce tyrosine phosphorylation of the 140-kda subunit of the interleukin 4 receptor. *J Biol Chem* **270**, 966-970.

SMITH, J.A. (1994) Neutrophils, host defense, and inflammation: a double- edged sword. *J Leukocyte Biol* **56**, 672-686.

SPITS, H. AND DE WAAL MALEFYT, R. (1992) Functional characterization of human IL-10. *Int. Arch. Allergy Immunol.* **99**, 8-15.

STERANKA, L.R., MANNING, D.C., DEHAAS, C.J., FERKANY, J.W., BOROSKY, S.A., CONNOR, J.R., VAVREK, R.J., STEWART, J.M., AND SNYDER, S.H. (1988) Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic actions. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 3245-3249.

STEWART, J.M. (1992) Bradykinin antagonists: The state of the art. *Agents Actions* **38**, 546-550.

SUNDQVIST, G. AND LERNER, U.H. (1996) Bradykinin and thrombin synergistically potentiate interleukin 1 and tumour necrosis factor induced prostanoid biosynthesis in human dental pulp fibroblasts *Cytokine* **8**, 168-177.

SYMONS, J.D., AND DUFF, G.W. (1990) A soluble form of the interleukin 1 receptor produced by a human B cell line. *FEBS. Lett.* **272**, 133-136.

SZOLCSANYI, J., ANTON, F., REEH, P.W., AND HANDWERKER, H.O. (1988) Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. *Brain Res.* **446**, 262-268).

TALLEY, J.J. (1997) Selective inhibitors of cyclooxygenase-2. *Expert Opinion on Therapeutic Patent.* **7**, 55-62

TE VELDE, A.A., HUIJBENS, R.J., HEIJE, K., DE VRIES, J.E., AND FIGDOR, C.G. (1990) Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. *Blood* **76**, 1392-1397.

TEIXEIRA, M.M., WILLIAMS, T.J., AND HELLEWELL, P.G. (1993) Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.* **110**, 1515-1521.

THORNBERRY, N.A., BULL, H.G., CALAYCAY, J.R., CHAPMAN, K.T., HOWARD, A.D., KOSTURA, M.J., MILLER, D.K., MOLINEAUX, S.M., WEIDNER, J.R., AUNINS, J., *ET AL* (1992) A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* **356**, 768-774.

TIFFANY, C.W. AND BURCH, R.M. (1989) Bradykinin stimulates tumor necrosis factor and interleukin-1 release from macrophages. *FEBS Lett.* **247**, 189-192.

TOGO, J., BURCH, R.M., DE HAAS, C.J., CONNOR, J.R., AND STERANKA, L.R. (1989) D-Phe7-substituted peptide bradykinin antagonists are not substrates for kininase II. *Peptides* **10**, 109-112.

TONUSSI, CR. AND FERREIRA, SH (1992) rat knee-joint incapacitation test: an objective screen for central and peripheral analgesics. *Pain* **48**, 421-427.

VAN HEES, J., AND GYBELS, J.M. (1972) Pain related to single afferent C-fibres from human skin. *Brain Res.* **48,** 397-400.

VANE, J.R. AND BOTTING, R.M. (1996) Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol.* 9-21.

VANNIER, E., MILLER, L.C., AND DINARELLO, C.A. (1992) Coordinated antiinflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 4076-4080.

VANNIER, E., MALEFYT, R.D., SALAZARMONTES, A., DEVRIES, J.E., AND DINARELLO, C.A. (1996) Interleukin-13 (IL-13) induces IL-1 receptor antagonist gene expression and protein synthesis in peripheral blood mononuclear cells: Inhibition by an IL-4 mutant protein. *Blood* **87**, 3307-3315.

VAVREK, R.J. AND STEWART, J.M. (1985) Competitive antagonists of bradykinin. *Peptides* **6**, 161-164).

VIGERS, G.P., CAFFES, P., EVANS, R.J., THOMPSON, R.C., EISENBERG, S.P., AND BRANDHUBER, B.J. (1994) X-ray structure of interleukin-1 receptor antagonist at 2.0-A resolution. *J. Biol. Chem.* **269**, 12874-12879.

VITA, N., LEFORT, S., LAURENT, P., CAPUT, D., AND FERRARA, P. (1995) Characterization and comparison of the interleukin 13 receptor with the interleukin 4 receptor on several cell types. *J Biol Chem* **270**, 3512-3517.

WANG, J.F., KHASAR, S.G., AHLGREN, S.C., AND LEVINE, J.D. (1996) Sensitization of C-fibres by prostaglandin E(2) in the rat is inhibited by guanosine 5'-O-(2-thiodiphosphate), 2'.5'-dideoxyadenosine and Walsh inhibitor peptide. *Neuroscience* **71**, 259-263.

WARD, P.E., BENTER, I.F., DICK, L., AND WILK, S. (1990) Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase M. *Biochem. Pharmacol.* **40**, 1725-1732.

WATKINS, L.R., WIERTELAK, E.P., GOEHLER, L.E., SMITH, K.P., MARTIN, D., AND MAIER, S.F. (1994) Characterization of cytokine-induced hyperalgesia. *BRAIN RES,*. **654**, 15-26.

WEBB, M., MCINTYRE, P., AND PHILLIPS, E. (1994) B1 and B2 bradykinin receptors encoded by distinct mRNAs. *J. Neurochem.* **62**, 1247-1253.

WEINREICH, D. (1986) Bradykinin inhibits a slow spike afterhyperpolarization in visceral sensory neurones. *Eur. J. Pharmacol.* **132**, 61-63.

WHALLEY, E.T., CLEGG, S., STEWART, J.M., AND VAVREK, R.J. (1987) The effect of kinin agonists and antagonists on the pain response of the human blister base. *Naunyn Schmiedebergs Arch. Pharmacol.* **336**, 652-655.

WIRTH, K., BREIPOHL, G., STECHL, J., KNOLLE, J., HENKE, S., AND SCHOLKENS, B. (1991) DesArg9-D-Arg(Hyp3, Thi5, D-Tic7, Oic8)bradykinin (desArg^{10}-(HOE140)) is a potent bradykinin B1 receptor antagonist. *Eur. J. Pharmacol.* **205**, 217-218.

WOOLF, C.J. (1987) Physiological, inflammatory and neuropathic pain. *Adv. Tech. Stand. Neurosurg.* **15**, 39-62.

XIONG, W., CHEN, L.M., AND CHAO, J. (1990) Purification and characterization of a kallikrein-like T-kininogenase. *J. Biol. Chem.* **265**, 2822-2827.

ZEITLIN, I.J. AND SMITH, A.N. (1973) Mobilization of tissue kallikrein in inflammatory disease of the colon. *Gut.* **14**, 133-138.